ACYCLOVIR LOADED SELF-ASSEMBLED pH SENSITIVE NANOPARTICLE AS A NOVEL DRUG CARRIER FOR TARGETING OF VIRAL INFECTION

A Dissertation Submitted to

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(Pharmaceutics)

Submitted by

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MELMARUVATHUR - 603 319

MAY 2012

CERTIFICATE

This is to certify that the dissertation entitled **"ACYCLOVIR LOADED SELF-ASSEMBELED P^H SENSITIVE NANOPARTICLE AS A NOVEL DRUG CARRIER FOR TARGETING OF VIRAL INFECTION"** Submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the award of the Degree of the Master of Pharmacy was carried out by **R. LOGESH (Register No. 26106004)** in the Department of Pharmaceutics under my direct guidance and supervision during the academic year 2011-2012.

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This is to certify that the dissertation entitled **"ACYCLOVIR LOADED SELF-ASSEMBELED P^H SENSITIVE NANOPARTICLE AS A NOVEL DRUG CARRIER FOR TARGETING OF VIRAL INFECTION"** is the bonafide research work carried out by **R.LOGESH (Register No. 26106004)** in the Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under the guidance of **Mr. T. AYYAPPAN, M.Pharm.,** Assistant Professor, Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, during the academic year 2011-2012.

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R.LOGESH

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ABBREVIATIONS

EC ---- Ethyl cellulose

PLA ---- Poly lactic acid

PLGA ---- Poly lactic glycolic acid

PCL ---- Poly capro Lactone

PHB ---- Poly hydroxyl butyrate

ESD ---- Emulsification solvent diffusion

UV ---- Ultra Violet

µg ---- Microgram

λ_{max} ---- Absorption maximum

ml ---- Millilitre

mg ---- Milligram

nm ---- Nanometer

FTIR ---- Fourier Transform Infra Red Spectroscopy

DSC ---- Differential Scanning Calorimetry

cm ---- Centimeter

% ---- Percentage

RH ---- Relative Humidity

I P ---- Indian Pharmacopoeia

t ---- Time

ICH ---- International Conference on Harmonization

gm ---- Grams

rpm ---- Revolutions per Minute

S.No. ---- Serial Number

Fig ---- Figure

°C ---- Degree Celsius

GIT ---- Gastrointestinal Tract

eg ---- Example

Eq ---- Equation

edn ---- Edition

NP ---- Nanoparticles

PCS ---- Photon correlation spectroscopy

ppm ---- parts per million

NDDS ---- Novel Drug Delivery System

M ---- Slope, Units of response

N ---- Normality

HCl ---- Hydro Chloric acid

F ---- Formulation

SLNP ---- Solid Lipid Nanoparticles

S.D ---- Standard Deviation

SEM ---- Scanning Electron Microscopy

KBr ---- Potassium Bromide

INTRODUCTION

1.INTRODUCTION

1.1. Novel Drug Delivery System:

(Chein Y.W., 2002; Bandyopadhyay A.K., 2008; Vyas S.P., 2009)

For many decades, the treatment of an acute disease or a chronic illness has been mostly accomplished by the delivery of drugs to the patients using various pharmaceutical dosage forms such as tablets, capsules, pills, suppositories, creams, ointments, aerosols and injectable. Even today these conventional drug delivery systems are the primary pharmaceutical products commonly seen in the prescriptions and which are available as over- the- counter medicines. The method by which a drug is delivered can have a significant effect on its efficacy. The slow progress in the treatment of certain disease has suggested a growing need for a multidisciplinary approach to the delivery of drugs to their target sites. Recently, several technical advancements have resulted in the development of new techniques which are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and or targeting the delivery of drug to a tissue. These are referred to as novel drug delivery system.

And they have revolutionized the method of medication, provides a number of therapeutic benefits. These drug delivery systems are based interdisciplinary approach that combines polymer science, pharmaceutics, bioconjugated chemistry and molecular biology.

1.1.1. Advantages of Novel Drug Delivery Systems:

(Shoba Rani R Hire math., 2008)

™ Improved therapy by increasing the duration of action and reduced side effects.

™ Increase the patient compliance through decreased dosing frequency and convenient routs of administration.

™ Achieve targeting of drugs to a specific site to reduce unwanted side effects and obtain maximum efficacy.

™ Lead to reduction in dose and thus reduction in side effects of drugs.

1.2. Rational For Targeted Drug Delivery System:

(Shoba Rani R Hire math., 2008)

Most of the drug introduced to clinical medicines produce their effects by interacting with cell and cell membrane-related structures and function through concentration-dependent, reversible interaction at a specific receptor sites. Obviously, to obtain the desirable therapeutic response, the correct amount of drug should be transported and delivered to the site of action with subsequent control of the drug input rate. The efficacy of many drugs is often limited by their potential to reach their therapeutic site of action. In most cases, only a small amount of the administered dose of the drug actually reaches this site, while most of the drug is distributed to the rest of the body depending on the physiochemical and biochemical properties of the drug.

Most drugs, when administered in conventional or controlled release dosage forms, freely travel throughout the body, leading to uptake by cells, tissue or organs other than those that contain their pharmacological receptors. This distribution of the drug to other tissues is unnecessary, wasteful and a potential cause of toxicity.

This lack of target specificity can be attributed to the formidable barriers that the body presents to a drug. A drug taken orally must withstand large fluctuations in pH as well as the action of enzymes before it get absorbed. Moreover, it needs to survive inactivation by the hepatic first pass effect. To produce a therapeutic effect, the drug must selectively access and interaction with the pharmacological receptors, and the concentration of the drug at the active site must be adequate.

1.3. Nanotechnology: *(Shoba Rani R Hire math., 2008)*

Nanoparticles are solid colloidal particles ranging 10-1000nm (1µm) in size. They consist of macromolecular in which the active ingredient (drug or biological active material) is dissolved, entrapped or encapsulated, and/or adsorbed or attached. Depending on the process used for the preparation, two types of nanoparticle are obtained: nanospheres and nanocapsules.

Nanospheres have a matrix-type structure in which the drug is dispersed, whereas nanocapsule exhibits a membrane-wall structure with an oily core containing drug. As nanoparticulate systems have a very high surface area, drug may also absorb on their surface. Nanoparticles are made of artificial or natural polymer. The use of these polymers is often restricted by their biocompatibility.

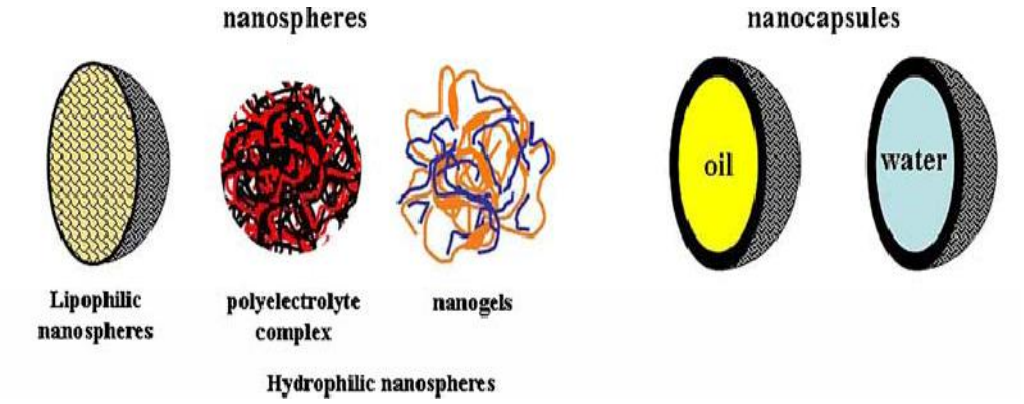


Fig. 1.1: Structure of nanospheres and nanocapsules

After preparation, nanoparticles are usually dispersed in liquid. Such a system can be administered to humans for example by injection, by the oral route, or used in ointments and ocular products. Alternatively, nanoparticles can be dried to a powder, which allows pulmonary delivery or further processing to tablets or capsules.

In drug delivery, nanoparticles should readily be biocompatible (not harmful for humans) and biodegradable (deteriorate and expulse in the body conditions). These properties, as well as targeting and controlled release, can be affected by nanoparticle material selection and by surface modification. Materials such as synthetic polymers, proteins or other natural macromolecules are used in the preparation of nanoparticles.

1.3.1. Advantages of Nanoparticles:

™ Enhancement of therapeutic effectiveness of the drug i.e. the overall pharmacological response per unit dose in increased.

™ Toxicity and adverse drug interactions are reduced to a possible extent.

E.g. polymethacrylic nanoparticles for targeting anticancer drug of doxorubicin to reduce liver toxicity. Nanoparticles posses' better stability as compared to liposome's which make it more important for many modes of targeting.

™ Nanoparticles formulated as amorphous offer solubility than standard crystalline formulations, thus improving the poor aqueous solubility of the drug and hence the bioavailability.

™ The methods of preparation are simple, easier and reproducible.

™ A high degree of patient compliance can be achieved.

™ Wide range of polymer can be used depends on the nature of the drug and usage i.e. biodegradable polymer for shorter periods, and non-biodegradable for longer periods.

™ It can easily pass through syringe needle and exhibit good rheological properties.

1.3.2. Limitations of Nanoparticles:

™ Their small size and large surface area can lead to particles aggregation, making physical handling of nanoparticles difficult in liquid and drug formulations.

™ In addition, small particle size and large surface area readily result in limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available.

1.3.3. Advantages over Microparticles: ***(****Ronald J. Neufeld., 2006)*

™ They have higher intracellular uptake compared to micro particles.

™ They are better suited for I.V. delivery since the smallest blood capillaries in then body is about 5-6µm.

1.3.4. Advantages over Liposomes:

 They have better stability in biological fluids and during storage.

 Their preparation is more amenable to scale up.

They have the unique ability to create a controlled release product.

1.4. Types of Nanoparticles: ***(****Pushpendra Goswami., 2011)*

™ **Fullerenes: Bucky balls and carbon tubes:**

Both members of the fullerene structural class, bucky balls and carbon tubes are carbon based lattice-like, potentially porous molecules.

™ **Liquid crystals:**

Liquid crystal pharmaceuticals are composed of organic liquid crystal materials that mimic naturally-occurring biomolecules like proteins or lipids. They are considered a very safe method or drug delivery and can target specific areas of the body where tissues are inflamed, or where tumors are found.

™ **Liposomes:**

Liposomes are lipid-based liquid crystals, used extensively in the pharmaceutical and cosmetic industries because of their capacity for breaking down inside cells once their delivery function has been met. Liposome's were the first engineered nanoparticles used for drug delivery but problems such as their propensity to fuse together in aqueous environments and release their payload, have lead to replacement, or stabilization using newer alternative nanoparticles. Liposomal formulations are the first NanoPharmaceuticals introduced to market, Doxil® PEGylated liposomal formulation for doxorubicin is the first product based on liposomes.

™ **Nanoshells:**

Also referred to as core-shells, nanoshells are spherical cores of a particular compound surrounded by a shell or outer coating of another, which is a few nanometers thick

™ **Quantum dots:**

Also known as nanocrystals, quantum dots are nanosized semiconductors that, depending on their size, can emit light in all colours of the rainbow. These nanostructures confine conduction band electrons, valence band holes, or excitons in all three special directions. Examples of quantum dots are semiconductor nanocrystals and core shell nanocrystals, where there is an interface between different semiconductor materials. They have been applied in biotechnology for cell labeling and imaging, particularly in cancer imaging studies.

™ **Superparamagnetic nanoparticles:**

Superparamagnetic molecules are those that are attracted to a magnetic field but do not retain residual magnetism after the field is removed. Nanoparticles of iron oxide with diameters in the 5-100 nm range have been used for selective magnetic bioseparations. Typical techniques involve coating the particles with antibodies to cell-specific antigens, for separation from the surrounding matrix.

™ **Dendrimers:**

Dendrimers are highly branched structures gaining wide use in nanomedicine because of the multiple molecular "hooks" on their surfaces that can be used to attach cell-identification tags, fluorescent dyes, enzymes and other molecules. The first dendritic molecules were produced around 1980, but interest in them has blossomed more recently as biotechnological uses are discovered.

™ **Nanorods:**

Typically 1-100 nm in length, nanorods are most often made from semiconducting materials and used in nanomedicine as imaging and contrast agents. Nanorods can be

made by generating small cylinders of silicon, gold or inorganic phosphate, among other materials.

1.5. Polymers Employed as Nanoparticle: *(Gadad A., 2011)*

1.5.1. Natural polymers used for the preparation of nanoparticles:

- **Proteins:**
  - Gelatin
  - Albumin
  - Lectins
  - Legumin
  - Vicilin
- **Polysaccharides**:
  - Alginate
  - Dextran
  - Chitosan
  - Agarose
  - Pullulan

1.5.2. Synthetic polymers used for the preparation of nanoparticles:

- **Pre-polymerized:**
  - Poly (ε – caprolactone)
  - Poly (lactic acid)
  - Poly (lactide-co-glycolide)
  - Polystyrene

 Polymerized in process:

- Poly (isobutylcyanoacrylates)
- Poly (butylcyanoacrylates)
- Poly (hexylcyanoacrylates)
- Poly methyl (methacrylate)

1.6. Preparation Techniques of Nanoparticles:

1.6.1. Synthetic performed polymers: *(Ronald J. Neufeld., 2006; Behera A.L., 2010)*

A. Emulsification/solvent evaporation:

Emulsification-solvent evaporation involves two steps. The first step requires emulsification of the polymer solution into an aqueous phase (see Fig. 1.2). During the second step polymer solvent is evaporated, inducing polymer precipitation as nanospheres.

A polymer organic solution containing the dissolved drug is dispersed into nanodroplets, using a dispersing agent and high-energy homogenization, in a nonsolvent or suspension medium such as chloroform (ICH, class 2) or ethyl acetate (ICH, class 3). The polymer precipitates in the form of nanospheres in which the drug is finely dispersed in the polymer matrix network. The solvent is subsequently evaporated by increasing the temperature under pressure or by continuous stirring. The size can be controlled by adjusting the stirring rate, type and amount of dispersing agent, viscosity of organic and aqueous phases, and temperature. Even though different types of emulsions may be used, oil/water emulsions are of interest because they use water as the nonsolvent, this simplifies and thus improves process economics, because it eliminates the need for recycling, facilitating the washing step and minimizing agglomeration.

However, this method can only be applied to liposoluble drugs, and limitations are imposed by the scale-up of the high energy requirements in homogenization. Frequently used polymers are PLA, PLGA, ethylcellulose (EC), cellulose acetate phthalate, poly (E-caprolactone) (PCL) and poly (hydroxybutyrate) (PHB). Drugs or model drugs encapsulated were albumin, texanus toxoid, testosterone, loperamide, prazinquantel, cyclosporin A, nucleic acid, and indomethacin.

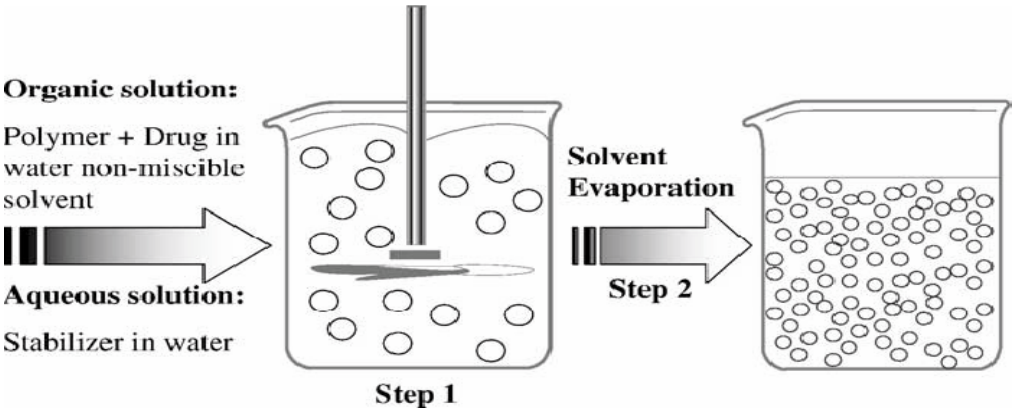


Fig. 1.2: Schematic representation of the emulsification-evaporation technique

B. Solvent displacement and interfacial deposition:

Solvent displacement and interfacial deposition are similar methods based on spontaneous emulsification of the organic internal phase containing the dissolved polymer into the aqueous external phase (see Fig. 1.3). However, solvent displacement forms nanospheres or nanocapsules, whereas interfacial deposition forms only nanocapsules.

Solvent displacement involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium in the presence or absence of a surfactant. The polymer, generally PLA, is dissolved in a water-miscible solvent of intermediate polarity, leading to the precipitation of

nanospheres. This phase is injected into a stirred aqueous solution containing a stabilizer as a surfactant. Polymer deposition on the interface between the water and the organic solvent, caused by fast diffusion of the solvent, leads to the instantaneous formation of a colloidal suspension. To facilitate the formation of colloidal polymer particles during the first step of the procedure, phase separation is performed with a totally miscible solvent that is also a nonsolvent of the polymer.

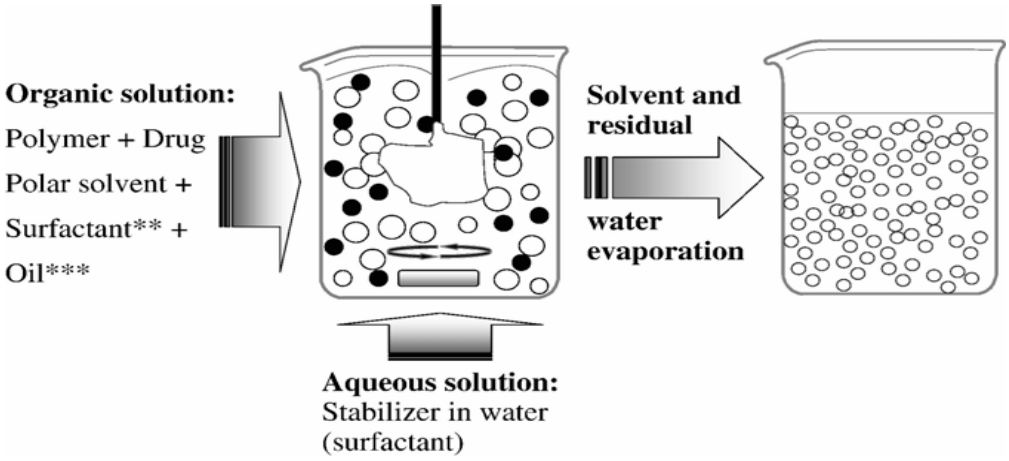


Fig. 1.3: Schematic representation of the solvent displacement technique

The solvent displacement technique allows the preparation of nanocapsules when a small volume of nontoxic oil is incorporated in the organic phase. Considering the oil-based central cavities of the nanocapsules, high loading efficiencies are generally reported for lipophilic drugs when nanocapsules are prepared. The usefulness of this simple technique is limited to water-miscible solvents, in which the diffusion rate is enough to produce spontaneous emulsification. Then, even though some water-miscible solvents produce certain instability when mixed in water, spontaneous emulsification is not observed if the coalescence rate of the formed droplets is sufficiently high. Although, acetone/dichloromethane (ICH, class 2) are used to dissolve and increase the entrapment of drugs, the dichloromethane increases

the mean particle size and is considered toxic. This method is basically applicable to lipophilic drugs because of the miscibility of the solvent with the aqueous phase, and it is not an efficient means to encapsulate water soluble drugs.

C. Emulsification/solvent diffusion:

Emulsification/solvent diffusion (ESD) was proposed in the literature based on the use of organic solvents, and then it was adapted to the following salting-out procedure. The encapsulating polymer is dissolved in a partially water soluble solvent such as propylene carbonate (ICH not given) and saturated with water to ensure the initial thermodynamic equilibrium of both liquids. In fact, to produce the precipitation of the polymer and the consequent formation of nanoparticles, it is necessary to promote the diffusion of the solvent of the dispersed phase by dilution with an excess of water when the organic solvent is partly miscible with water or with another organic solvent in the opposite case. Subsequently, the polymer-water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion to the external phase and the formation of nanospheres or nanocapsules, according to the oil-to-polymer ratio. Finally, the solvent is eliminated by evaporation or filtration, according to its boiling point. The procedure is illustrated in Fig. 1.4.

This technique presents several advantages, such as high encapsulation efficiencies (generally N70%), no need for homogenization, high batch-to-batch reproducibility, ease of scale-up, simplicity, and narrow size distribution. Disadvantages are the high volumes of water to be eliminated from the suspension and the leakage of water-soluble drug into the saturated-aqueous external phase

during emulsification, reducing encapsulation efficiency. As with some of the other techniques, this one is efficient in encapsulating lipophilic drugs

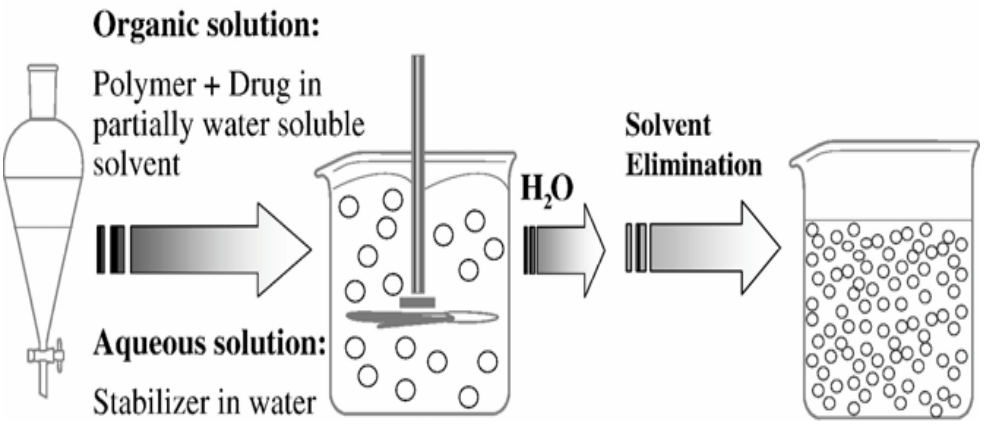


Fig. 1.4: Schematic illustration of the ESD technique

Several drug-loaded nanoparticles were produced by the ESD technique, including mesotetra(hydroxyphenyl) porphyrin-loaded PLGA (p-THPP) nanoparticles, doxorubicin-loaded PLGA nanoparticles, plasmid DNA-loaded PLA nanoparticles, coumarin-loaded PLA nanoparticles, indocyanine, cyclosporine (Cy-A)-loaded gelatin and cyclosporin (Cy-A)-loaded sodium glycolate nanoparticles.

D. Salting out with synthetic polymers:

Salting-out is based on the separation of a water miscible solvent from aqueous solution via a salting-out effect. The salting-out procedure can be considered as a modification of the emulsification/solvent diffusion. Polymer and drug are initially dissolved in a solvent such as acetone, which is subsequently emulsified into an aqueous gel containing the salting-out agent (electrolytes, such as magnesium chloride, calcium chloride, and magnesium acetate, or non- electrolytes such as sucrose) and a colloidal stabilizer such as polyvinylpyrrolidone or hydroxyethylcellulose. This oil/water emulsion is diluted with a sufficient volume of

water or aqueous solution to enhance the diffusion of acetone into the aqueous phase, thus inducing the formation of nanospheres. The selection of the salting out agent is important, because it can play an important role in the encapsulation efficiency of the drug. Both the solvent and the salting-out agent are then eliminated by cross-flow filtration.

This technique used in the preparation of PLA, poly- (methacrylic) acid, and EC nanospheres leads to high efficiency and is easily scaled up. The main advantage of salting out is that it minimizes stress to protein encapsulants. Salting out does not require an increase of temperature and, therefore, may be useful when heat sensitive substances have to be processed. The greatest disadvantages are exclusive application to lipophilic drugs and the extensive nanoparticle washing steps. The preparative steps of this procedure are described in Fig. 1.5.

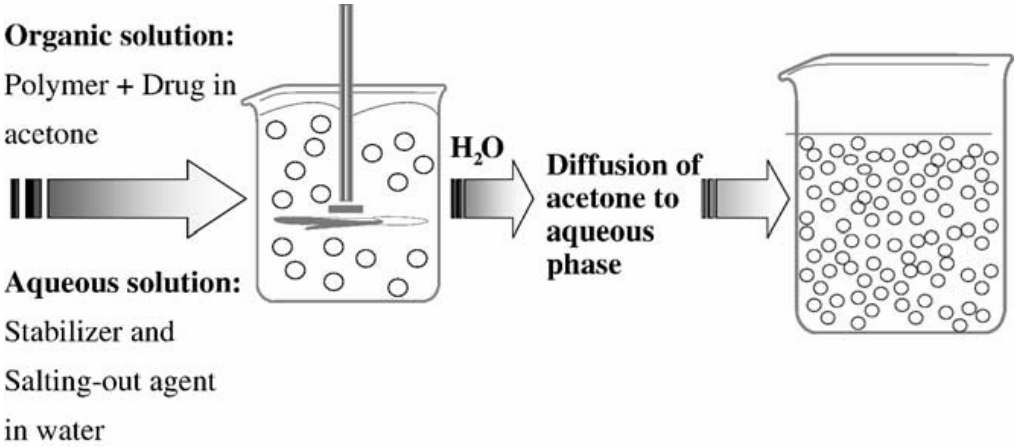


Fig. 1.5: Schematic diagram of the salting-out technique

1.6.2. Production of nanoparticles from natural macromolecules:

A. Albumin nanoparticles produced in an external-oily emulsion:

Two main methods are used in the preparation of albumin microspheres, characterized by the method of stabilization, thermal treatment at elevated

temperatures (958–1708ºC) or chemical treatment in vegetable oil, iso-octane emulsions, or aqueous medium. Other techniques involve slight modification of either of the two methods In this case albumin nanospheres were formed by homogenizing the oil phase containing the albumin droplets and thermally stabilized by heating at 1758 - 1808ºC for 10 minutes. This mixture was cooled and diluted with ethyl ether to reduce the viscosity of the oil phase to permit separation by centrifugation.

Heat treatment of albumin is applicable only to drug molecules that are not heat sensitive. For this reason, nanoparticles were produced emulsifying serum albumin aqueous solution in cottonseed oil at 258ºC, then denaturing the albumin by resuspending the particles in ether containing the cross-linking agents 2,3-butadiene or formaldehyde. The particles were stirred, isolated by centrifugation, and dried by lyophilization. Particles released the drug doxorubicin much faster than particles formed by heat treatment, but the purification step remains the main problem with the elimination of the cottonseed oil. A technique was proposed based on the desolvation of natural macromolecules, which simplifies the purification step.

As a modification of this method, an aqueous solution of albumin was emulsified in chloroform containing hydroxypropylcellulose and EC as stabilizers. The emulsified macromolecule is subsequently cross-linked with glutaraldehyde and washed. Because of the need for chlorinated solvents, this technique does not offer much advantage over the other techniques.

B. Gelatin nanoparticles produced in an external-oily emulsion:

Emulsified gelatin solution droplets were hardened by cooling the emulsion below the gelation point in an ice bath, resulting in gelation of the gelatin droplets.

Gelled nanodroplets were filtered, washed, and cross-linked with formaldehyde. The particle size ranged between 100 and 600 nm with a mean of 280 nm. This technique is applicable to heat-sensitive drugs; however, a number of drugs can be covalently bound to the gelatin by formaldehyde treatment, which constitutes a disadvantage. Additionally, cross-linking increases significantly the size of the particles. Furthermore, a significant disadvantage of the cross-linking agent relates to its toxicity, and this point must be carefully considered. In this context, it would be of interest to study carefully the influence of different formulation and process parameters in this process according to the application.

Another interesting system for drug delivery systems could be nanoparticulate carriers from bioacceptable macromolecules. For this reason, vegetable protein fractions termed gliadins have been chosen from wheat gluten, to efficiently encapsulate lipophilic substances such as a-tocopherol. Gliadins possess the ability to interact with epidermal keratin as a result of their richness in praline; this property leads to a desired controlled release of drug.

C. Alginate nanoparticles:

Sodium alginate is a water-soluble polymer that gels in the presence of multivalent cations such as calcium. Alginate particles are usually produced by drop wise extrusion of sodium alginate solution into calcium chloride solution. Alginate particle size depends on the size of the initial extruded droplet. The smallest particles produced had a minimum size of 1 to 5 Am, obtained by air atomization. The preparation of alginate nanoparticles was first achieved in a diluted aqueous sodium alginate solution in which gelation was induced by the addition of a low concentration of calcium.

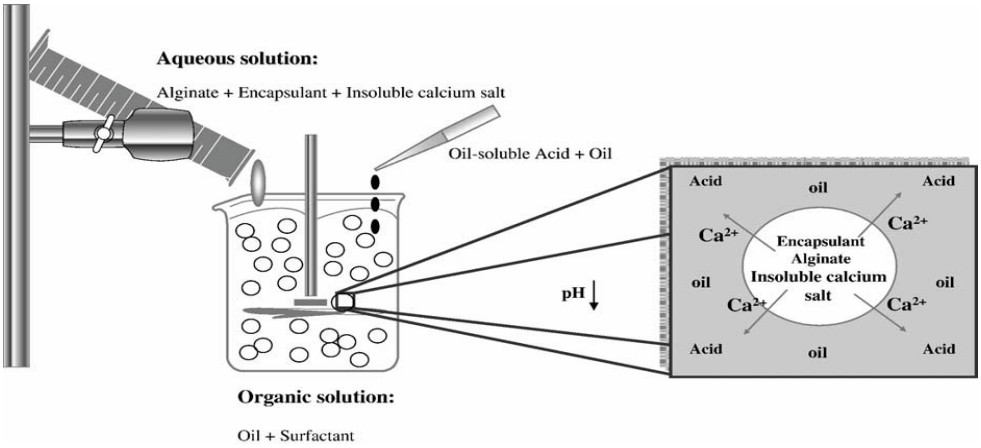


Fig. 1.6: Schematic representation of the emulsification–internal gelation technique using alginate.

This leads to the formation of invisible clusters of calcium alginate gels. In an additional advance, alginate particles have been produced by using a modified emulsification/internal gelation method. The preparation of alginate nanoparticles via this method does not require specialized equipment and can be performed at ambient temperature. The main difficulty of this method is the nanoparticle washing step to eliminate the residual oil droplets, but new strategies have been devised.

D. Chitosan nanoparticles:

Chitosan nanoparticles have been developed to encapsulated proteins such as bovine serum albumin, tetanus and diphtheria toxoid, vaccines, anticancer agents, insulin, and nucleic acids. Chitosan considerably enhanced the absorption of peptides such as insulin and calcitonin across the nasal epithelium.

The methods proposed to prepare chitosan nanoparticles are based on the spontaneous formation of complexes between chitosan and polyanions or the gelation of a chitosan solution dispersed in an oil emulsion. Various methods for producing chitosan nanoparticles are described in the literature.

Chitosan nanoparticles obtained by formation of a spontaneous complex between chitosan and polyanions such as tripolyphosphate have small diameters (200–500 nm) and show a quasi spherical shape under transmission electron microscopy. Chitosan nanoparticles produced by a promoting gelation in an emulsification-based method as illustrated in Fig. 1.7, results in a diameter of 400 nm.

Compared with the previously described method, this technique has a major disadvantage of involving organic solvents during the isolation of the particles; these are difficult to remove and may cause toxicity.

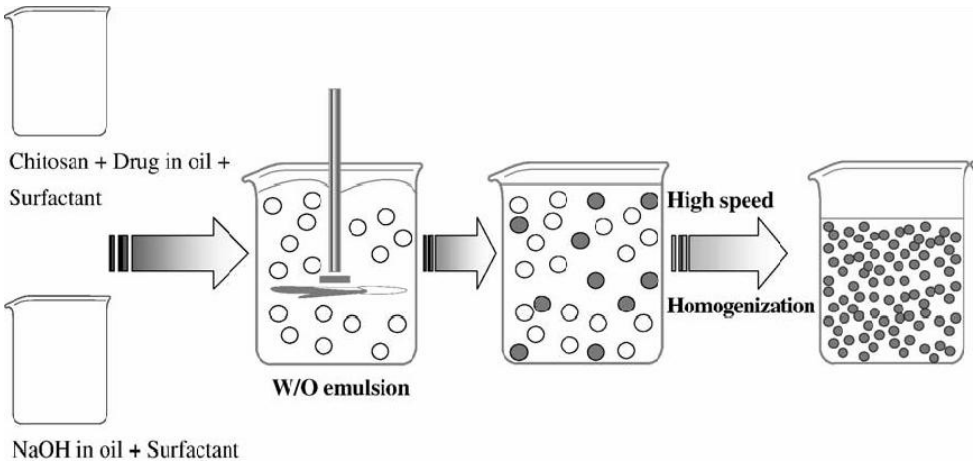


Fig. 1.7: Schematic representation of chitosan nanoparticles preparation by the emulsification technique

E. Agarose nanoparticles:

Agarose nanoparticles were developed for the administration of therapeutic proteins and peptides. Agarose aqueous solution forms thermally reversible hydrogels while being cooled below the gelling temperature (318–368ºC). Thermal gelation results from the formation of helicoidal structures responsible for a three-dimensional network in which large amounts of water can be entrapped. The hydrogel, being

hydrophilic, inert, and biocompatible, forms a suitable matrix for proteins and peptides that can be entrapped in the gel during formation.

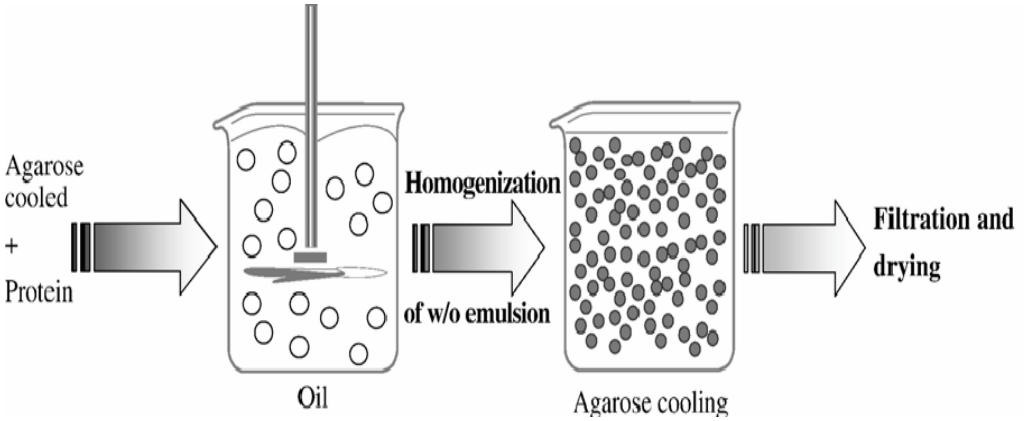


Fig. 1.8: Schematic representation of agarose nanoparticles preparation by the emulsification technique

This methodology requires the preparation of an agarose solution in corn oil emulsion at 408ºC. Peptides and proteins to be encapsulated are initially added to the agarose solution. The small size of the dispersed aqueous nanodroplets is achieved by homogenization. Gelation of agarose is then induced by diluting the emulsion with cold corn oil under agitation at 58ºC. The liquid nanodroplets then gel to protein-containing agarose hydrogel nanoparticles.

1.7. Characterization of Nanoparticles: *(Gadad A., 2011; Mohanraj V. J., 2006)*

Table 1.1: Characterization of nanoparticles

S.NO	PARAMETERS	METHODS
1	Particle size and morphology	Transmission electron microscopy, scanning (electron, force, tunneling) microscopy freeze-fracture electron microscopy, Photon-correlation spectroscopy.
2	Surface charge Surface hydrobhobicity	Zeta potential measurement Hydrophobic interaction chromatography Contact angle measurement
3	Density	Helium compression pyenometry Density gradient centrifugation
4	Molecular weight Crystallinity	Gel permeation chromatography X-ray diffraction, Differential scanning calorimetory
5	Surface charge Surface hydrobhobicity	Contact angle measurements Hydrophobic interaction chromatography Zeta potential measurement
6	Carrier drug interactions	DSC
7	Nanoparticle dispersion stability	Critical flocculation temperature(CFT)
8	Surface Chemical analysis	secondary ion mass spectroscopy x- ray photoelectron spectroscopy nuclear magnetic resonance Fourier transform infrared spectroscopy
9	Release profile	In-vitro release characteristics under physiologic and sink conditions
10	Drug stability	Bioassay of drug extracted from nanoparticle chemical analysis of drug

A. Freeze-drying: *(Saez A., 2000; Randolph TW., 1999)*

Briefly, in freeze-drying (lyophilization), solvent (e.g. Water) in the system is frozen followed by its sublimation in vacuum. At present, freeze-drying is the method of choice to remove water from the system in nanoparticle formulations and also avoids instability problem of liquid preparation if it is done effectively. However, freeze-drying of nanoparticles is a complicated process because the stability of the colloidal nanoparticle dispersion in most cases disturbed leading to aggregation before the particulate population is dry. Several variables affect the final result: freezing rate and temperature, drying temperatures, pressure, use of excipients like bulking agents or cryo- and lyoprotectants and changes in pH or ionic strength. After successful drying, one should be able to re-disperse particles in their initial dispersing medium without causing significant changes in their size and drug content.

Cryoprotectants protect the product during freezing, whereas lyoprotectants provide stability during the drying step. Glycine, sucrose, glucose and poly (vinyl pyrrolidone) are Cryoprotectants, while disaccharides such as trehalose, sucrose lactose act usually as lyoprotectants.

B. Particle size:

Particle size and size distribution are the most important characteristics of nanoparticle systems. They determine the *in vivo* distribution, biological fate, toxicity and the targeting ability of nanoparticle systems. In addition, they can also influence the drug loading, drug release and stability of nanoparticles. Many studies have demonstrated that nanoparticles of sub-micron size have a number of advantages over microparticles as a drug delivery system.

Generally nanoparticles have relatively higher intracellular uptake compared to microparticles and available to a wider range of biological targets due to their small size and relative mobility. In a subsequent study, the nanoparticles penetrated throughout the submucosal layers in a rat in situ intestinal loop model, while microparticles were predominantly localized in the epithelial lining. It was also reported that nanoparticles can across the blood-brain barrier following the opening of tight junctions by hyper osmotic mannitol, which may provide sustained delivery of therapeutic agents for difficult-to-treat diseases like brain tumors. Tween 80 coated nanoparticles have been shown to cross the blood-brain barrier. In some cell lines, only submicron nanoparticles can be taken up efficiently but not the larger size microparticles.

Drug release is affected by particle size. Smaller particles have larger surface area, therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release. Whereas, larger particles have large cores which allow more drug to be encapsulated and slowly diffuse out. Smaller particles also have greater risk of aggregation of particles during storage and transportation of nanoparticle dispersion. It is always a challenge to formulate nanoparticles with the smallest size possible but maximum stability. Polymer degradation can also be affected by the particle size. For instance, the rate of PLGA polymer degradation was found to increase with increasing particle size *in vitro*. It was thought that in smaller particles, degradation products of PLGA formed can diffuse out of the particles easily while in large particles, degradation products are more likely remained within the polymer matrix for a longer period to cause autocatalytic degradation of the polymer

material. Therefore, it was hypothesized that larger particles will contribute to faster polymer degradation as well as the drug release.

However, Panyam et al prepared PLGA particles with different size ranges and found that the polymer degradation rates *in vitro* were not substantially different for different size particles. Currently, the fastest and most routine method of determining particle size is by photon correlation spectroscopy or dynamic light scattering. Photon-correlation spectroscopy requires the viscosity of the medium to be known and determines the diameter of the particle by Brownian motion and light scattering properties. The results obtained by photon-correlation spectroscopy are usually verified by scanning or transmission electron microscopy (SEM or TEM)

C. Surface properties of nanoparticles:

When nanoparticles are administered intravenously, they are easily recognized by the body immune systems, and are then cleared by phagocytes from the circulation. Apart from the size of nanoparticles, their surface hydrophobicity determines the amount of adsorbed blood components, mainly proteins (opsonins). This in turn influences the *in-vivo* fate of nanoparticles. Binding of these opsonins onto the surface of nanoparticles called opsonization acts as a bridge between nanoparticles and phagocytes. The association of a drug to conventional carriers leads to modification of the drug biodistribution profile, as it is mainly delivered to the mononuclear phagocytes system (MPS) such as liver, spleen, lungs and bone marrow. Indeed, once in the blood stream, surface non modified nanoparticles (conventional nanoparticles) are rapidly opsonized and massively cleared by the macrophages of MPS rich organs. Generally, it is IgG, compliment C_3 components that are used for recognition of foreign substances, especially foreign macromolecules. Hence, to

increase the likelihood of the success in drug targeting by nanoparticles, it is necessary to minimize the opsonization and to prolong the circulation of nanoparticles *in-vivo*. This can be achieved by (a) surface coating of nanoparticles with hydrophilic polymers/surfactants; (b) formulation of nanoparticles with biodegradable copolymers with hydrophilic segments such as polyethylene glycol (PEG), polyethylene oxide, polyoxamer, poloxamine and polysorbate 80 (Tween 80). Studies show that PEG conformation at the nanoparticle surface is of utmost importance for the opsonin repelling function of the PEG layer. PEG surfaces in brush-like and intermediate configurations reduced phagocytosis and complement activation whereas PEG surfaces in mushroom-like configuration were potent complement activators and favoured phagocytosis 2.

The zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. Nanoparticles with a zeta potential above (+/-) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles. The zeta potential can also be used to determine whether a charged active material is encapsulated within the centre of the nanocapsule or adsorbed onto the surface.

D. Drug loading:

Ideally, a successful nanoparticulate system should have a high drug-loading capacity thereby reduce the quantity of matrix materials for administration. Drug loading can be done by two methods:

• Incorporating at the time of nanoparticles production (incorporation method)

• Absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution (adsorption /absorption technique).

Drug loading and entrapment efficiency very much depend on the solid-state drug solubility in matrix material or polymer (solid dissolution or dispersion), which is related to the polymer composition, the molecular weight, the drug polymer interaction and the presence of end functional groups (ester or carboxyl). The PEG moiety has no or little effect on drug loading. The macromolecule or protein shows greatest loading efficiency when it is loaded at or near its isoelectric point when it has minimum solubility and maximum adsorption. For small molecules, studies show the use of ionic interaction between the drug and matrix materials can be a very effective way to increase the drug loading.

E. Drug release:

The release of active ingredients is important in case nanospheres. Much theoretically possible mechanism may be considered for release of drugs from carriers.

They are

1. Osmatically driven burst mechanism.
2. Pore diffusion.
3. Erosion or degradation of polymer.

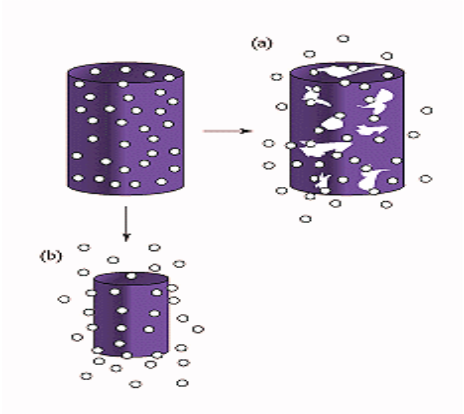


Fig. 1.9: Drug delivery from (a) bulk-eroding and (b) surface-eroding biodegradable System

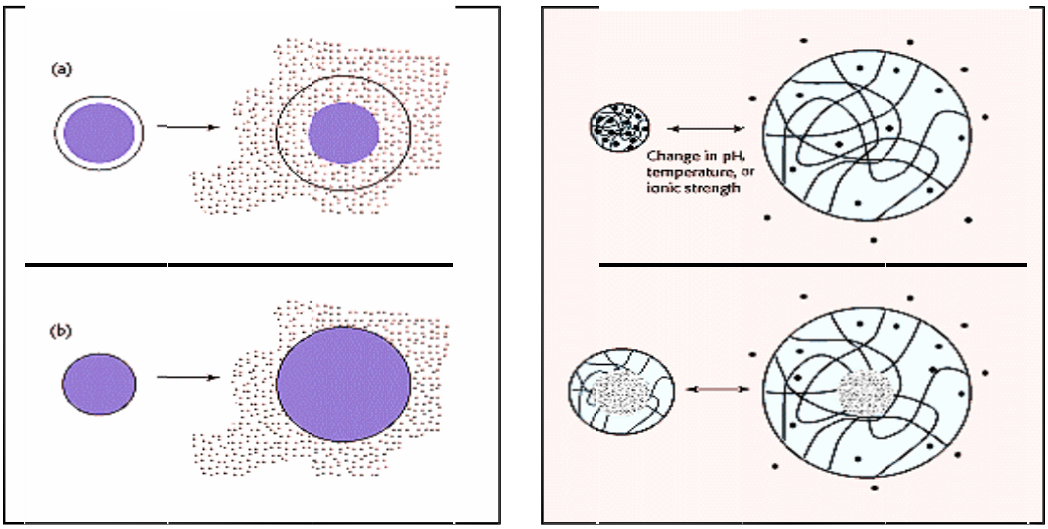


Fig. 1.10: Mechanism of drug release from polymer. (a) Reservoir (b) Matrix

To develop a successful nanoparticulate system, both drug release and polymer biodegradation are important consideration factors. In general, drug release rate depends on: (1) solubility of drug; (2) desorption of the surface bound/adsorbed drug; (3) drug diffusion through the nanoparticle matrix; (4) nanoparticle matrix erosion/degradation; and (5) combination of erosion/diffusion process. Thus solubility, diffusion and biodegradation of the matrix materials govern the release process. In the case of nanospheres, where the drug is uniformly distributed, the

release occurs by diffusion or erosion of the matrix under sink conditions. If the diffusion of the drug is faster than matrix erosion, the mechanism of release is largely controlled by a diffusion process.

The rapid initial release or 'burst' is mainly attributed to weakly bound or adsorbed drug to the large surface of nanoparticles. It is evident that the method of incorporation has an effect on release profile. If the drug is loaded by incorporation method, the system has a relatively small burst effect and better sustained release characteristics. If the nanoparticle is coated by polymer, the release is then controlled by diffusion of the drug from the core across the polymeric membrane. The membrane coating acts as a barrier to release, therefore, the solubility and diffusivity of drug in polymer membrane becomes determining factor in drug release.

Drug:

- ™ Molecular weight.
- ™ Physicochemical properties
- ™ Drug-carrier interaction
- ™ Diffusion ; desorption from the surface Particles
- ™ Type and amount of matrix material
- ™ Size and density of the particle
- ™ Extent and nature of any cross linking; denaturation of polymerization, presence of adjutants
- ™ Drug release profile desired.
- ™ Surface characterization such as charge and permeability.
- ™ Degree of biodegradation, biocompatibility and toxicity.
- ™ Total disintegration of particles

Environment:

- Hydrogen ion concentration
- Polarity
- Ionic strength
- Presence of enzymes
- Temperature

Furthermore release rate can also be affected by ionic interaction between the drug and addition of auxillary ingredients. When the drug is involved in interaction with auxillary ingredients to form a less water soluble complex, then the drug release can be very slow with almost no burst release effect; whereas if the addition of auxillary ingredients e.g. addition of ethylene oxide-propylene oxide block copolymer (PEO-PPO) to chitosan, reduces the interaction of the model drug bovine serum albumin (BSA) with the matrix material (chitosan) due to competitive electrostatic interaction of PEO-PPO with chitosan, then an increase in drug release could be observed.

Various methods which can be used to study the *in vitro* release of the drug are: (1) side-by-side diffusion cells with artificial or biological membranes; (2) dialysis bag diffusion technique; (3) reverse dialysis bag diffusion; (4) agitation followed by ultracentrifugation/centrifugation; (5) Ultra-filtration or centrifugal ultra-filtration techniques. Usually the release study is carried out by controlled agitation followed by centrifugation. Due to the time-consuming nature and technical difficulties encountered in the separation of nanoparticles from release media, the dialysis technique is generally preferred.

1.8. Methods for the Purification of Nanoparticles on the Laboratory Scale:

(Mathlowitz Edith., 2009)

Table 1.2: Purification methods of nanoparticles

S.No	Method	Drawback
1	Gel filtration	Removal of high molecular impurities difficult
2	Dialysis	Removal high molecular weight impurities difficult Scaling-up difficult
3	Ultra centrifugation	Aggregation of purities Time consuming process Scaling-up difficult

1.9. Therapeutic Application of Nanoparticle:

(Mohanraj V.J., 2006; Gadad A., 2011; Behera A.L., 2010)

A) Tumor targeting using nanoparticulate delivery systems:

The rationale of using nanoparticles for tumor targeting is based on following characteristics: 1) Nanoparticles will be able to deliver a concentrate dose of drug in the vicinity of the tumor targets via the enhanced permeability and retention effect or active targeting by ligands on the surface of nanoparticles. 2) Nanoparticles will reduce the drug exposure of healthy tissues by limiting drug distribution to target organ. Studies show that the polymeric composition of nanoparticles such as type, hydrophobicity and biodegradation profile of the polymer along with the associated drugs molecular weight, its localization in the nanospheres and mode of incorporation technique, adsorption or incorporation, have a great influence on the drug distribution pattern in vivo.

The exact underlying mechanism is not fully understood but the bio distribution of nanoparticles is rapid, within hour to 3 hours, and it likely involves mononuclear phagocytic system (MPS) and endocytosis/phagocytosis process. Such propensity of MPS for endocytosis/ phagocytosis of nanoparticles provide an opportunity to effectively deliver therapeutic agents to these cells. This biodistribution can be of benefit for the chemotherapeutic treatment of MPS-rich organs/tissues localized tumors like hepatocarcinoma, hepatic metastasis arising from digestive tract or gynecological cancers, bronchi pulmonary tumors, primitive tumors and metastasis, small cell tumors, myeloma and leukaemia.

B) Ligand attached nanoparticles:

To be successful as a drug delivery system, nanoparticles must be able to target tumors, which are localized outside MPS-rich organs. In the past decade, a great deal of work has been devoted to developing so called stealth particles or PEGylated nanoparticles, which are invisible to macrophages or phagocytes. A major breakthrough in the field came when the use of hydrophilic polymers (such as polyethylene glycol, poloxamines, poloxamers, and polysaccharides) to efficiently coat conventional nanoparticle surface produced an opposing effect to the uptake by the MPS. These coatings provide a dynamic cloud of hydrophilic and neutral chains at the particle surface, which repel plasma proteins. As a result, those coated nanoparticles become invisible to MPS, therefore remained in the circulation for a longer period of time and hence called as long circulating nanoparticles.

Hydrophilic polymers can be introduced at the surface in two ways, either by adsorption of surfactants or by use of block or branched copolymers for production of nanoparticles. Studies show nanoparticles containing a coat of PEG not only have a

prolonged half-life in the blood compartment but also be able to selectively extravagate in pathological sites such as tumors or inflamed regions with a leaky vasculature. As a result, such long-circulating nanoparticles have increased the potential to directly target tumors located outside MPS rich regions. A size less than 100 nm and a hydrophilic surface are essential in achieving the reduction of opsonisation reactions and subsequent clearance by macrophages. Coating conventional nanoparticles with Surfactants or PEG to obtain a long circulating carrier has now been used as a standard strategy for drug targeting *in-vivo*.

Extensive efforts have been devoted to achieving active targeting of nanoparticles in order to deliver drugs to the right targets, based on molecular recognition processes such as ligand receptor or antigen-antibody interaction. Considering that fact that folate receptors are over expressed on the surface of some human malignant cells and the cell adhesion molecules such as selectins and integrins are involved in metastatic events, nanoparticles bearing specific ligands such as folate may be used to target ovarian carcinoma while specific peptides or carbohydrates may be used to target integrins and selectins 46. Targeting with small ligands appears more likely to succeed since they are easier to handle and manufacture. Furthermore, it could be advantageous when the active targeting ligands are used in combination with the long-circulating nanoparticles to maximize the likelihood of the success in active targeting of nanoparticles.

C) Nanoparticles for oral delivery of peptides and proteins:

Significant advances in biotechnology and biochemistry have led to the discovery of a large number of bioactive molecules and vaccines based on peptides and proteins. Development of suitable carriers remains a challenge due to the fact that

bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and their susceptibility to gastrointestinal degradation by digestive enzymes. Polymeric nanoparticles allow encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic degradation. For instance, it has been found that insulin-loaded nanoparticles have preserved insulin activity and produced blood glucose reduction in diabetic rats for up to 14 days following the oral administration.

The surface area of human mucosa extends to 200 times that of skin. The gastrointestinal tract provides a variety of physiological and morphological barriers against protein or peptide delivery, e.g., (a) proteolytic enzymes in the gut lumen like pepsin, trypsin and chymotrypsin; (b) proteolytic enzymes at the brush border membrane (endopeptidases); (c) bacterial gut flora; and (d) mucus layer and epithelial cell lining itself. The histological architecture of the mucosa is designed to efficiently prevent uptake of particulate matter from the environment. One important strategy to overcome the gastrointestinal barrier is to deliver the drug in a colloidal carrier system, such as nanoparticles, which is capable of enhancing the interaction mechanisms of the drug delivery system and the epithelia cells in the GI tract.

D) Nanoparticles for gene delivery:

Polynucleotide vaccines work by delivering genes encoding relevant antigens to host cells where they are expressed, producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Such vaccines produce both humoral and cell-mediated immunity because intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the immune system. The key ingredient of polynucleotide vaccines, DNA, can be

produced cheaply and has much better storage and handling properties than the ingredients of the majority of protein-based vaccines. Hence, polynucleotide vaccines are set to supersede many conventional vaccines particularly for immunotherapy.

However, there are several issues related to the delivery of poly nucleotides, which limit their application. These issues include efficient delivery of the polynucleotide to the target cell population and its localization to the nucleus of these cells, and ensuring that the integrity of the polynucleotide is maintained during delivery to the target site. Nanoparticles loaded with plasmid DNA could also serve as an efficient sustained release gene delivery system due to their rapid escape from the degradative endolysosomal compartment to the cytoplasmic compartment. Hedley et al. reported that following their intracellular uptake and endolysosomal escape, nanoparticles could release DNA at a sustained rate resulting in sustained gene expression. This gene delivery strategy could be applied to facilitate bone healing by using PLGA nanoparticles containing therapeutic genes such as bone morphogenic protein.

E) Nanoparticles for drug delivery in to the brain:

The blood-brain barrier (BBB) is the most important factor limiting the development of new drugs for the central nervous system. The BBB is characterized by relatively impermeable endothelial cells with tight junctions, enzymatic activity and active efflux transport systems. It effectively prevents the passage of water soluble molecules from the blood circulation into the CNS, and can also reduce the brain concentration of lipid-soluble molecules by the function of enzymes or efflux pumps. Consequently, the BBB only permits selective transport of molecules that are essential for brain function. Strategies for nanoparticle targeting to the brain rely on

the presence of and nanoparticle interaction with specific receptor-mediated transport systems in the BBB. For example polysorbate 80 / LDL, transferring receptor binding antibody (such as OX26), lactoferrin, cell penetrating peptides and melanotransferrin.

Table 1.3: Therapeutic application of nanoparticles

S.No	Applications	Material	Purpose
1	Cancer therapy	poly(alkyl cyanoacrylates) nanoparticles with anti cancer agents, oligonucleotides	Targeting, reduce toxicity, enhanced uptake of anti tumor agents, improved *in-vitro* and *in-vivo stability*
2	Intercellular targeting	Poly(alkyl cyanoacrylates) poly ester nanoparticles with anti-parasitic or anti viral agents	RES for intra cellular infections
3	Prolonged systemic circulation	Polyesters with adsorbed PEGS or pluronics or derivatized polyesters	Prolonged systemic drug effects, avoid uptake by the RES
4	vaccine adjuvant	Poly(methacrylates) nanoparticles with vaccines	Enhanced immune response
5	Ocular delivery	Poly (alkyl cyanoacrylates) nanoparticles with steroids, anti inflammatory agents.	Improved retention of drug
6	DNA delivery	DNA–alginates nanoparticles DNA-chitosan nanoparticles	Enhanced delivery and significantly higher expression level
7	Other applications	Nanoparticles with adsorbed enzymes	Enzyme immunoassay , radio-imaging

1.10. Marketed Formulations of Nanoparticles: *(Nelson A Ochekpe., 2009)*

Table 1.4: Marketed formulations of nanoparticles

Type of Nanostructure	Trade name	Active ingredient	Indication	Company
Polymeric Nanoparticles	Adagen	Adenosine Deaminase	Adenosine deaminase (ADA) enzyme deficiency	Enzon Pharmaceuticals Inc., Bridgewater, NJ, USA
	Onscaspar	L-asparaginase	Acute lymphoblastic leukaemia	Enzon Pharmaceuticals Inc., NJ, USA
	Copaxone	Glatiramer Acetate	Relapsing-remitting multiple sclerosis	Tev Pharmaceuticals, Tikva, Isreal
	Macugen	Pegaptanib Sodium	All types of neovascular agerelated macular Degeneration	Nektar Therapeutics, San Carlos, CA, USA; OSI Pharmaceuticals, Melville, NY, USA
	Pegasys	Pegylated interferon alfa 2a	Hepatitis C	Nektar Therapeutics, CA, USA
	Neulasta	Pegfilgrastim	Neutopenia	Nektar Therapeutics, CA, USA; Amgen Inc, Thousand Oaks, USA.

1.11. Introduction to Antivirals: *(Don A. Ballington., 2005)*

There are fewer medications to treat viral infections than there are for bacterial infections. Part of the difficulty is that antibiotics often disrupt a cellular process that is unique to the bacterium being treated. This allows dosing of the medication without causing toxicity to the patient. However, because viruses use the cellular processes of the host to function and replicate, medications that block the life cycle of the virus are often toxic to the patient. Thus, the antiviral have been formulated to search and destroy the virus cell lodged in its host cell without interfering with the host cell's normal function. Antiviral are among the drugs of choice for the following conditions. Cytomegalovirus (CMV) retinitis

- genital herpes
- herpes simplex
- herpes simplex keratitis
- herpes zoster (shingles)
- influenza prophylaxis
- organ transplantation
- varicella (chicken pox)

1.12. Viruses and Their Characteristics:

A virus is a minute infection agent which is much smaller than a bacterium. Unlike a bacterium, a virus does not have all the components of a cell and thus it is able to replicate only within a living host cell. Viruses, among the most common infectious agents in humans, replicate by using the host cells' metabolic processes. A virus can infect a spectrum of cells including animal, plant, or bacteria cells. Most common viruses are spread by one of the following routes.

™ direct contact

™ ingestion of contaminated food and water

™ inhalation of airborne particles

The individual virus particle, a virion, consists of nucleic acid (nucleoid), either deoxyribonucleic acid (RNA) (but not both), and a protein shell (capsid) that surrounds and protects the nucleic acid. Depending on the virus, the capsid may be converted with spikes that attach to the host cell. Binding of the spikes to membrane receptors simulates a process whereby the cell engulfs the virus. A virus without an envelope covering the capsid is called a naked virus.

1.13. Stages of Viral Infection:

Within the body, viral infection takes place at the cellular level and in the following stages.

1. The virus attaches to a cell receptor

2. The cell membrane indents and closes around the virus (endocytosis) and thus the virus penetrates the cell

3. The virus escapes into cytoplasm

4. The virus uncoats, sheds its covering, and presents its DNA or RNA to cell nucleus.

5. This allows the virus to convert the nuclear activity in the cell to viral activity and rapidly reproduce new viral particles. (It user the energy of the host cell to infect the cell).

When viruses take over host cell nuclear activity, they synthesized viral enzymes, DNA, and protein, which lead to production of more virus particles. The infected host cells may be so damaged that it disintegrates, releasing bursts of mature visions are released slowly. During the release process, the virion often receives an

envelope, or capsid, from the nuclear or the plasma (cell) membrane. All virus-infected cells have some cellular characteristics different from those of uninfected cells. These differences provide opportunities to target and block viral division with medications without affecting normal cells.

1.14. Viral Classification:

Viral infections are classified in two ways. The first classification is the duration or length of time they have been present in the body as well as their severity. The second classification measures the extent of the infections within the body or the parts of the body that are affected.

1.14.1. Viral Duration and Severity:

With the classification of duration and severity there are three categories: acute, chronic, and slow. An acute viral infection quickly resolves with no latent infection. Examples include the common cold, influenza, and various other respiratory tract infections. A chronic viral infection has a protected course with long periods of remission interspersed with reappearance, such as the herpes virus infection. A slow viral infection maintains a progressive course over months or years, with cumulative damage to body tissue, ultimately ending in the host's death. Examples of this type of destructive viral infection include human immunodefficiency virus (HIV) and diseases affecting the central nervous system (CNS).

1.14.2. Viral Infection:

When evaluating the extent of the viral infection, it must be determined whether the infection is local or generalized. A local viral infection affects tissue of a single system, such as the respiratory tract. A generalized viral infection is one that

has spread or is spreading to other tissue by way of the bloodstream or tissue of the CNS.

1.15. Viral Life Cycle: *(Ilango K., 2005)*

Viral life cycle varies with species, but they all share a general petten can be sequenced as follows:

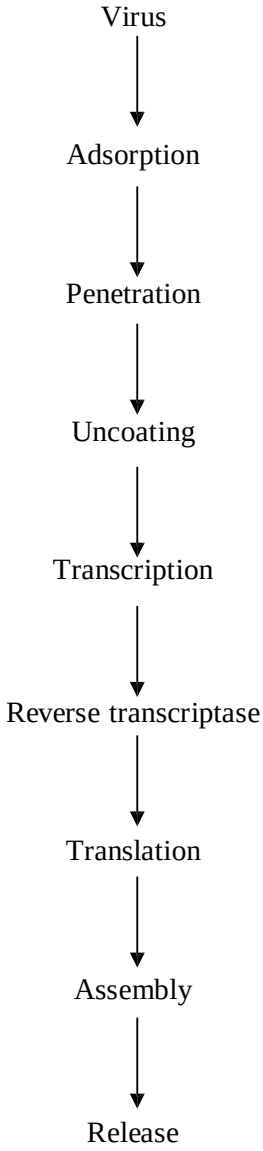


Fig. 1.11: Pathology of viral life cycle

1) Adsorption: Attachment of virus to the host cell.

2) Penetration: Penetration of virus into the cell.

3) Uncoating: The genetic material or viral genome (DNA or RNA) passes into the host -cell, leaving the capsid covering outside the host cell.

4) Transcription: Production of viral mRNA from the viral genome.

5) Translation: Viral genome enters the cytoplasm or nucleo plasma and directs or utilizes the host nucleic acid machinery for the synthesis of new viral protein and for the production of more viral genome. The viral protein modifies the host cell and allows the viral genome to replicate by using host and viral enzyme. This is often the stage at which the cell is irreversible modified and eventually killed.

6) Assembly of viral particle: New viral coat protein assembly into capsid and viral genomes.

7) Release of the mature virus from the cell by budding process or rupture of the cell and repeat of the process, in fresh host cell. Since the host cell machinary is totally utilized for production of new visions, the normal cell function ceases at the times of replication.

General approaches for treating the virus infection by antiviral agents are:

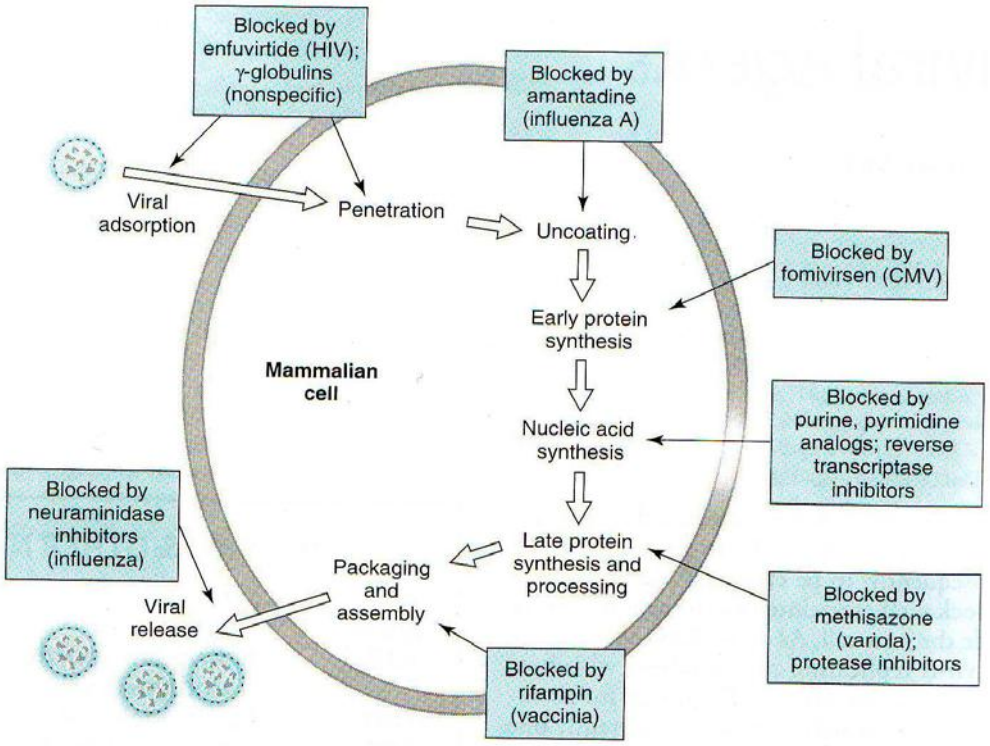


Fig. 1.12: Various classes of anti-viral agents against the virus life cycle

a) Interference of virus attachment to the host

b) Inhibition of virus associated enzymes

c) Inhibition of transcription process

d) Inhibition of translation process

e) Interference with viral regulatory process

f) Interference with glycosylation, phosphorylation, etc.,

g) Interference with viral assembly of viral protein

h) Interference with release of virus from cell surface membrane.

Table 1.5: List of some RNA virus types together with disease that they cause

RNA Viruses	
Virus	**Disease**
Picornaviruses	Polio, Hepatitis A
Rhinovirus	Commen cold, Pneumonia
Togavirus	Rubella, Encephalitis
Flavirus	Yellow fever, Dengue fever, St.Louis encephalitis
Bunyaviruses	Encephalitis, Hemorrhagic fever
Rhabdoviruses	Vesicular stomatitis
Myxoviruses	Mumps, Measles
Reoviruses or Rotaviruses	Diarrhea
Arenaviruses	Lymphatic choriomeningitis
Retroviruses	Human immunodeficiency syndrome (HIV)

Table 1.6: List of some DNA virus types together with disease that they cause

DNA Viruses	
Virus	**Disease**
Herpes viruses	Herpes, Cold sores
Papovaviruses	Polyoma, warts
Adenoviruses	Respiratory complications
Poxvirus	Smallpox
Parvirus	Canine distemper

LITRATURE SURVEY

2. LITERATURE SURVEY

2.1. Literature Review:

Recent advancement in nanoparticle drug delivery systems:

1) Adlin jino nesalin j. *et al.*, (2009) nanoparticles with Flutamide using chitosan polymer by ionic gelation technique. Nanoparticles of different core: coat ratio were formulated and analyzed for total drug content, loading efficiency, particle size and *in vitro* drug release studies. From the drug release studies it was observed that nanoparticles prepared with chitosan in the core: coat ratio 1:4 gives better sustained release for about 12 hrs as compared to other formulations.

2) Anilkumar J. Shinde. *et al.*, (2011) was formulated nanoparticle with simvastatin. Simvastatin is a lipid lowering agent, and BCS class-II drug having low solubility and high permeability. Since simvastatin undergoes extensive first pass extraction in the liver, the availability of the drug to the general circulation is low (< 5%). Nanoparticles were prepared by precipitation-solvent deposition method using 3^2 full factorial design. From the preliminary trials, the constraints for independent variables X1 (amount of PLGA) and X2 (amount of Pleuronic F-68) have been fixed. The prepared formulations were further evaluated for drug content, in vitro drug release pattern, short term stability and drug excipient interactions. The application of factorial design gave a statistically systematic approach for the formulation and optimization of nanoparticles with desired particle size and high entrapment efficiency. Drug: polymer ratio and concentration of stabilizer were found to

influence the particle size and entrapment efficiency of simvastatin loaded PLGA nanoparticles. These results indicate that simvastatin loaded PLGA nanoparticles could be effective in sustaining drug release for a prolonged period.

3) Arpita Bhattacharya. *et al.*, (2003) work was focused for development by generalized upgradable bench top level technology for nanocapsulation of antineoplastic drug substances. Methotrexate was selected as a modal drug and calcium alignate was used as a coating material. A nebulizer head was so designed as to atomize a drug-polymer micro emulsion directly into calcium chloride cross linker media. Nanocapsules thus formed were compared with those produced by in situ gelling technique. Control of various process parameters resulted in the development of superior drug nanocapsules by pneumatic nebulisation technique with good yield.

4) Atmaram P. pawer. *et al.*, (2009) were prepared Eudragit®-EPO based nanoparticle suspension of isolated andrographolide in order to increase its solubility and efficacy. Andrographolide nanoparticle was prepared by precipitation technique. The 3^2 factorial design was used to study the effect of Eudragit®-EPO and Pluronic® F-68 on characteristics of nanoparticle suspension. There was marked increase in drug dissolution with complete drug release within 10 minutes in nanoparticle suspension and redispersed nanoparticle suspension compare to pure drug. However, lyophilization retarded the drug release. The results were confirmed by histopathological studies on hepatic lesions. Thus particle engineering can we used to improve pharmaceutical properties of phytoconstituents.

5) Bivash Mandal. *et al.*, (2010) was aimed to prepare polymeric nanosuspension with inert polymer resin (Eudragit RL100) to the improving the availability of sulfacetamide at the intraocular level to combat bacterial infections. Nanosuspensions

were prepared by the solvent displacement method using acetone and Pluronic F108 solution. Drug to polymer ratio was selected as formulation variable. Characterization of the nanosupension was performed by measuring particle size, zeta potential, Fourier Transform infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Powder X-Ray Diffraction (PXRD), drug entrapment efficiency and *in vitro* release. In addition, freeze drying, redispersibility and short term stability study at room temperature and at 40ºC were performed. The results indicate that the formulation of sulfacetamide in Eudragit RL100 nanosuspension could be utilized as potential delivery system for treating ocular bacterial infections.

6) Debra T. Auguste. *et al.*, (2008) were investigated pH-Sensitive poly(N,N-dimethylaminoethyl methacrylate (DMAEMA)/2-hydroxyethyl methacrylate (HEMA) nanoparticles. It was prepared for the triggered release of paclitaxel within a tumor microenvironment. Tumors exhibit a lower extracellular pH than normal tissues. We show that paclitaxel release from DMAEMA/HEMA particles can be actively triggered by small, physiological changes in pH (within 0.2 to 0.6 pH units). Monodispersed nanoparticles were synthesized by forming an O/W emulsion followed by photopolymerization. Cell viability studies indicate that pHsensitive DMAEMA/HEMA nanoparticles are not cytotoxic and may be used as an efficient, feedback-regulated drug delivery carrier.

7) Gambhire Makarand. *et al.*, (2010) was studied the preparation and physic-chemical evaluation of Rifampicin loaded poly-(lactic-co-glycolic) acid (PLGA) nanoparticles as per 3^2 Factorial Design are presented. PLGA (X1) and PVA (Polyvinyl alcohol) solution (X2) as a stabilizing agent were used as independent variables where Particle size (PS) (Y1), Entrapment Efficiency (EE) (Y2) and % Drug

Release at 12^{th} hour (REL) (Y3) were taken as dependant variables. Rifampicin nanoparticles were prepared by multiple emulsion solvent evaporation method. Hence, this investigation demonstrated the potential of the experimental design in understanding the effect of the formulation variables on the quality of rifampicin nanoparticles.

8) Gambhire M. S. *et al.*, (2011) prepared the formulation of simvastatin (SIMVA) loaded solid lipid nanoparticles by pre-emulsion ultrasonication technique. The variables drug: lipid ratio, percentage of lipid phase surfactant and sonication time were studied at three levels and arranged in a Box-behnken design, to study the influence on response variables particle size and % entrapment efficiency (%EE). From the statistical analysis of data, polynomial equations were generated. The physical characteristics of SIMVA-SLN were evaluated using particle size analyzer, differential scanning calorimetry and X-ray diffraction. The *in-vitro* drug release study of SIMVA-SLN using modified Franz diffusion cell showed significantly low release of simvastatin (37.08%) than dispersion of pure drug (97.2 %).

9) Mahendra nakarani. *et al.*, (2010) studied about the Itraconazole nosuspension in order to increase the aqueous solubility and improve its formulation related parameters, dissolution and hence oral bioavailability. Itraconazole nanosuspension was prepared by pearl milling technique using zirconium oxide beads as a milling media, poloxamer 407 as a stabilizer and gycerol as a wetting agent. Effect of various process parameters like, stirring time and ratio of the beads were optimized by keeping Drug:surfactant:milling media (1:3:50) as a constant initially then optimized process parameters were used to optimize formulation parameters by 3^2 factorial designs. Optimized nanosuspension showed a mean particle diameter of 294 nm,

spherical shape with surface oriented surfactant molecules, which were stabilized formulation, high drug content, no chemical instability and no significant change in crystalline nature after formulation also. The *in vitro* dissolution profile of optimized formulation compared to pure drug and marketed formulation (Canditral Capsule) using 0.1N hydrochloric acid as dissolution medium showed higher drug release compared to the pure drug and marketed formulation.

10) Panchaxari M Dandagi. *et al.*, (2011) was developed nanoparticle for oral controlled release of clotrimazole and to improve its bioavailability. Nanoparticles of Clotrimazole were prepared by emulsion-solvent evaporation technique using PLGA as polymer. Different formulations were prepared using drug and polymer in 1:1, 1:2, 1:3 ratios respectively. There was a striking improvement in the relative and absolute bioavailability of drug. The oral administration of unencapsulated drug was detected in various body organs such as liver, lungs and spleen up to 6 hours. In case of drug was found in liver, lungs, spleen, small intestine and kidney for one day. In can be concluded that oral administration of clotrimazole nanoparticles from a sound basis for controlling drug release and improving its bioavailability.

11) Panchaxari M Dandagi. *et al.*, (2011) was prepared and evaluated the polymeric biodegradable nanoparticles of Etoposide. To overcome the inherent drawback associated with conventional drug delivery of Etoposide. An attempt is being made to design an alternative drug delivery system in form of nanoparticles. By Formulated Etoposide in nanoparticles using PLGA, we can reduce toxicity, enhanced therapeutic index and modify pharmacokinetics and tissue distribution. In the present study polymeric biodegradable nanoparticles(NPs) of Etoposide (ETP) were prepared by modified spontaneous emulsification solvent diffusion method using polylactic-co-

glycolic acid (PLGA) as biodegradable matrix. The formulation were then characterized with respect to size and its surface morphology, zeta potential, entrapment efficiency, *in-vitro* drug release profile, sterility testing, stability studies and in vivo tissue distribution study. In conclusion, the drug loaded nanoparticles showed preferential drug targeting to lungs followed by liver, kidney and spleen. Stability study indicated that 4ºC is the most suitable temperature for storage of PLGA nanoparticles. Etoposide loaded nanoparticle is endowed with several exclusive advantages and hence holds potential for further research and clinical application.

12) Ping Li. *et al.*, (2008) were studied the Chitosan-alginate (CS /ALG) nanoparticles prepared by ionotropic pre-gelation of an alginate core followed by chitosan polyelectrolyte complexation technique. Nifedipine was chosen as a model drug. Morphology and structure characterization of nanoparticles were investigated by transmission electron microscope (TEM) and Fourier transform infrared spectra (FTIR), respectively. The diameter of the nanoparticles was about 20-50 nm, suitable for uptake within the gastrointestinal tract due to their nanosize range and mucoadhesive properties. Nifedipine released from chitosan–alginate nanoparticles was 26.52% at pH1.5, 69.69% at pH6.8 and 56.50% at pH7.4 within 24hour. This suggests that the release of nifedipine from nanoparticles was pH-responsive.

13) Rosario Pignatello. *et al.*, (2006) study focused to improve the stability of cloricromene (AD6) in ophthalmic formulations and its drug availability at the ocular level. To this end, AD6-loaded polymeric nanoparticle suspensions were made using inert polymer resins (Eudragit RS100 and RL100). We modified the quasi-emulsion solvent diffusion technique by varying some formulation parameters (the drug-to-

polymer ratio, the total drug and polymer amount, and the stirring speed). The chemical stability of AD6 in the nanosuspensions was assessed by preparing some formulations using (unbuffered) isotonic saline or a pH 7 phosphate buffer solution as the dispersing medium. The obtained nanosuspensions showed mean sizes and a positive surface charge (zeta-potential) that make them suitable for an ophthalmic application; these properties were maintained upon storage at 4°C for several months. In vitro dissolution tests confirmed a modified release of the drug from the polymer matrixes. Nanosuspensions prepared with saline solution and no or lower amounts of surfactant (Tween 80) showed an enhanced stability of the ester drug for several months, with respect to an AD6 aqueous solution. Based on the technological results, AD6-loaded Eudragit Retard nanoparticle suspensions appear to offer promise as a means to improving the shelf life and bioavailability of this drug after ophthalmic application.

14) Selvaraj. S. *et al.,* (2010) were investigated the topical application of Acyclovir as eye ointment remains a concern for effective management of various ocular viral diseases owing to poor ocular drug bioavailability. Hence the study was aimed to develop and evaluate nanosphere colloidal suspension containing Acyclovir as potential ophthalmic drug delivery system. The Acyclovir loaded chitosan nanoparticles were prepared by ionic gelation of chitosan with tripolyphosphate anions (TPP). The nanoparticles were characterised by Scanning Electron Microscopy (SEM), Zeta potential analyser, Differential Scanning Calorimetry (DSC) and Fourier Transform InfraRed (FTIR) Spectroscopy. The invitro diffusion profile of acyclovir from the nanoparticles showed a sustained release of the drug over a period of 24 hours. Kinetic release profiles of acyclovir from nanoparticles appeared to fit best

with Higuchi model with zero order and the non-Fickian diffusion was superior phenomenon. Thus the results suggest that Acyclovir loaded chitosan nanoparticle suspension appears promising for effective management of ocular viral infections.

15) Shashidhar Kerur. *et al.*, (2009) was aimed to formulated a novel ophthalmic nanosuspension (ONS), an alternative carrier system to traditional colloidal carriers for controlled release (CR) of acyclovir (ACV). In the present study, ONS is employed to avoid some of major disadvantages of colloidal carriers systems such as instability in culdesac and short half life by increasing efficiency of drug encapsulation as well as by CR. A quassi-emulsion solvent evaporation method was used to prepare ACV loaded Eudragit RS 100 ONS with the aim of improved ocular bioavailability and distribution. The release profile revealed from best formulation followed Non-Fickian diffusion mechanism. Stability studies showed a maximum drug content and almost similar in vitro release compared to the initial data found for the sample stored at 4°C. Overall, the study also revealed that ONS was capable of releasing the drug for a prolonged period of time and increased bioavailability.

16) Sivabalan M. *et al.*, (2011) was formulated and evaluated chitosan and Eudragit® nanoparticles of 5- Fluorouracil for cancer therapy. Nanoparticles of 5-Fluorouracil were prepared using chitosan , Eudragit® S 100, liquid paraffin and Tween -20 using Emulsion droplet coalescence method. The concentration of the polymers Chitosan and Eudragit® S 100 were selected based on the results on preliminary screening. The nanoparticles prepared were evaluated for morphology, loading efficiency, invitro release and invitro anticancer activities. The particle shape and morphology of the prepared 5-Fluorouracil nanoparticles were determined by SEM analysis. A franz diffusion cell was used to monitor 5-Fluorouracil release from

the nanoparticles. The *in-vitro* anticancer activity of formulated nanoparticles was carried out in Dalton-Lymphoma ascites (DLA) cells bearing mice. The formulations CF1, CF2, EF2 and EF3 showed good drug release from the polymer. Among the four formulations EF 2 (1% Chitosan & 1.5 % Eudragit® S 100) showed maximum drug release in 12 hours diffusion study and good entrapment efficiency. *In-vitro* anticancer study revealed that the formulated nanoparticles were found to have good cidal activity on cancer cells in sustained manner.

17) Robhash Kumar Subedi. *et al.*, (2009) were studied the Solid lipid nanoparticles (SLN) loaded with doxorubicin. It was prepared by solvent emulsification-diffusion method. Glyceryl caprate (Capmul®MCM C10) was used as lipid core, and curdlan as the shell material. Dimethyl sulfoxide (DMSO) was used to dissolve both lipid and drug. Polyethylene glycol 660 hydroxystearate (Solutol®HS15) was employed as surfactant. Major formulation parameters were optimized to obtain high quality nanoparticles. In conclusion, SLN with small particle size, high EE, and relatively high DL for doxorubicin can be obtained by this method.

18) Suganeswari M. *et al.*, (2011) was aimed to prepare, characterize and evaluate nanoparticles containing Hypolipidaemic drug (Atorvastatin calcium: D1N) Antihypertensive agent (Amlodipine besylateD2N) loaded by nanoprecipitation method using tribloere polymeric stabilizer (Pluronic F68). Biodegradable nanoparticles formulated from poly (D, L-lactide-co-glycolide) (PLGA) polymers are being extensively investigated for various drug delivery applications. Nanoparticles using PLGA polymers were formulated using nanoprecipitation technique, and were characterized for size, drug loading, and in vitro release. The Amlodipine has potency to promote the activity of Atorvastatin. Therefore, Atorvastatin and Amlodipine

combination was taken for this research. A biodegradable nanoparticulate approach was introduced here with a view to improving the efficacy and safety of Atorvastatin calcium. Particulate systems like nanoparticles have been used as a physical approach to alter and pharmacodynamic properties of various types of drug molecules. The nanoparticluate suspension of Amlodipine is to improve its absorption rate and therapeutic efficacy.

19) Umasankar K. *et al.*, (2010) were formulated flutamide nanoparticles. Flutamide is an oral antiandrogen drug primarily used to treat prostate cancer. The purpose of this research is to minimize the frequency of doses and toxicity and to improve the therapeutic efficacy by formulating flutamide nanoparticle. Flutamide nanoparticles were formulating by solvent evaporation method using Methacrylic acid copolymer (RL100) with three different ratios. Nanparticles were characterized by determining its particle size, drug entrapment efficiency, drug release and stability studies. The particle size ranged between 335 nm to 620 nm. Drug content was found of be supportive to the drug release pattern. The invitro release of flutamide nanoparticles were carried out which exhibited a sustained release of flutamide from nanoparticles upto 16 hours. The results showed that nanoparticles were more beneficial in providing drug delivery system.

20) Vandana Singh. *et al.*, (2010) published the article related to Application of nanotechnology in drug delivery system has opened up new areas of research in sustained release of drugs. The nanoparticles have the advantages of reaching otherwise less accessible sites in the body by escaping phagocytosis and entering tiny capillaries. Sustained release of the drug from the nanoparticles could maintain the therapeutic concentration for long durations. Rosiglitazone loaded gelatin

nanoparticles were prepared by two step desolvation method. The nanoparticles were characterized for various parameters. The results indicated that two step desolvation method is well suited to prepare gelatin nanoparticles and the process variables of the procedure can be fine tuned depending on the clinical applications.

21) Vikas jain. *et al.*, (2011) studies about mucoadhesive nanosuspension of ciprofloxacin and it was designed in order to improve the solubility, bioavailability and efficacy for the treatment of typhoid fever. The identity and purity of drug was established. The compatibility of drug with various excipients was ascertained by FTIR techniques, which indicated no interaction between the drug and excipients. Four different formulations were prepared by optimizing various parameters using different polymers like soya lecithin, pluronic F-68, polyvinyl alcohol, and polyvinylpyrrolidone K30. Particle size and polydispersity index were determined by photon correlation spectroscopy. The promising formulations prepared from combination of soya lecithin and pluronic F68 and those based on soya lecithin alone were subjected to dissolution profile studies. The later formulation exhibited fast dissolution rate as compared to the former. Thus nanosuspension based on soya lecithin was incorporated into hydrogels prepared using different grades of carbopol 934 and 971 as mucoadhesive polymers. After 10 hours, mucoadhesive nanosuspensions showed 45 to 56% release. The developed mucoadhesive nanosuspensions exhibited satisfactory physical stability. The studies indicated potential of these formulations as novel gastro retentive systems.

22) Vikram M. Pandya. *et al.*, (2011) literature on poor water solubility and slow dissolution rate are issues for the majority of upcoming and existing biologically active compounds. Simvastatin is poorly water-soluble drug and its bioavailability is

very low from its crystalline form. The purpose of the present investigation was to increase the solubility and dissolution rate of simvastatin by the preparation of nanosuspension by nanoprecipitation technique at laboratory scale. Prepared nanosuspension was evaluated for its particle size and in vitro dissolution study and characterized by differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). These results indicate the suitability of 2^3 factorial design for preparation of simvastatin loaded nanosuspension significantly improved in vitro dissolution rate, and thus possibly enhance fast onset of therapeutic drug effect.

23) Yadav A V. *et al.*, (2009) was prepared and characterized the Eudragit E 100 Nanoparticles of Carvedilol. Nanoparticles of Carvedilol with Eudragit E 100 were prepared by the nanoprecipitation method using polymeric stabilizer Poloxamer 407. nanoparticles of Carvedilol were obtained with high encapsulation efficiency. Nanoparicles of Carvedilol were obtained with high encapsulation efficiency (85-91%). The drug release from the carvedilol nanoparticles showed within 5 minutes. These studies suggest that the feasibility of formulating carvedilol-loaded Eudragit E 100 nanoparticles for the treatment of hypertension.

24) Yadav S. *et al.*, (2011) was developed the sustained release mucoadhesive microspheres of Acyclovir. Sodiumcarboxymethylcellulose and hydrxypropylmethylcellulose were used as mucoadhesive polymers. The microspheres were prepared using solvent evaporation technique. The effect of variable concentration of polymers on the characteristics of the microspheres was studied. The use of higher amounts of polymer significantly increased the median size of the microspheres. The efficiency of encapsulation increased when the concentration of polymers was increased. The poor bioavailability of acyclovir is

attributed to short retention of its dosage form at the absorption sites. The results of mucoadhesion study showed better retention of Sodium CMC microspheres (8.0±0.8 h) in duodenal and jejunum regions of intestine. Overall, the result indicated prolonged delivery with significant improvement in oral bioavailability of acyclovir from mucoadhesive microspheres due to enhanced retention in the upper GI tract.

25) Yiguang Jin. *et al.*, (2006) studied about designing of Self-assembled drug delivery systems (SADDS). It was prepared from the amphiphilic conjugates of hydrophilic drugs and lipids through self-assembling into small-scale aggregates in aqueous media. The outstanding characteristic of SADDS is that they are nearly wholly composed of amphiphilic prodrugs. The self-assembled nanoparticles (SAN) as one of SADDS had been prepared from the lipid derivative of acyclovir (SGSA) in the previous paper. They were further studied on the properties and the in vitro/in vivo behavior in this paper. The SAN kept the physical state stable upon centrifugation or some additives including some inorganic salts, alkaline solutions, surfactants and liposomes except for HCl solution, CaCl2 solution and animal plasma. Autoclave and bath heat for sterilization hardly influenced the SAN. However, gamma-irradiation strongly destroyed the structure of SAN and SGSA was degraded.

26) Zhang guoliang. *et al.*, (2006) aimed to improve the cancer-targeting and selective activity of antineoplastic agent [5-fluorouracil (5-FU)]. It is a novel pH-responsive drug delivery system [pullulan acetate/sulfonamide (PA/SDM) conjugate] was synthesized by a diafiltration method. Sulfonamide was grafted to the hydrophobically modified pullulan acetate to enhance the pH sensitivity for better cancer-targeting delivery. 5-FU was loaded into the self-assembled nanoparticles by the same method. The drug-loaded self-assembled nanoparticles were successfully

obtained and characterized in terms of particle size, morphology and drug loading and release profile at various pHs. The results showed that the mean diameter of the self-assembled particles was approximately 100 nm, with uniform size and good spherical morphology. The nanoparticles showed good stability at pH 7.4, which is equal to that of the normal body fluid, but shrank and aggregated below pH 6.8, which is close to the pH with tumors. The loading efficiency and concentration of released 5-FU was monitored at 269 nm on the UV/Vis spectrophotometer. The release profile was heavily pH-dependent around physiological pH, and the release rate was significantly enhanced under pH of 6.8.

2.2. DRUG PROFILE

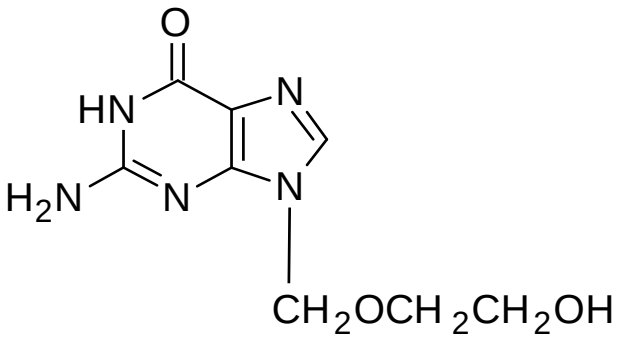
ACYCLOVIR: *(IP., 2007; Merck Index., 1997; USP., 2009; Tripathi K.D., 2004)*

Acyclovir is active against herpes group of virus; H. *simplex* type 1 is the most sensitive followed by the H. *simplex* type II> varicella-zoister =Epstein-bar virus; Cytomegalovirus (CMV) is practically not affected .the prototype antiviral agent used to treat various types of herpes infections. Since, acyclovir was the first antiviral to be considered the gold standard for the treatment of herpes infections, all other anti herpes virus medications are compared to it. It is approved for the prophylaxis of herpes genitals.

A. Chemical name:

9 – [(2 hydroxyethoxy) methyl] -9H- guanine 2-amino-1, 9-dihydro-9-[(2-hydroxyethoxy) methyl]-6H-purin-6-one

B. Structural formula:



C. Physical properties:

- **Molecular formula** : $C_8H_{11}N_5O_3$
- **Molecular weight** : 225
- **Description** : White, crystalline powder, fine powder
- **Odour** : Characteristic
- **Taste** : Bitter to alkaline
- **Melting point** : Melt at about 230º with decomposition (BP)
  : Melt at about 250º with decomposition (USP)
- **Dissociation constant (pKa):** 2.3, 9.2
- **Solubility** : Freely soluble in dimethyl sulphoxide;
  Slightly soluble in water;
  Very slightly in ethanol (95%);
  Dissolves in dilute solutions of mineral acids and Alkaline hydroxides.
- **Storage** : Store in well closed containers

D. Mode of action:

Acyclovir is a synthetic analogue deoxy guanosine in which the carbohydrate moiety is acyclic. The modes of action of acyclovir consist of the following consecutive mechanism:

1. Conversion to active acyclovir monophosphate within cells by viral thymidine kinase. This phosphorylation reaction occurs faster in infected cell than normal cell, because acyclovir is a poor substrate for the normal cell thymidine kinase. Acyclovir monophosphate is further converted in to di and triphosphate by a normal cellular enzyme guanosine monophosphate kinase.

2. Acyclovir triphosphate inhibits viral DNA polymerase by competing deoxy guanosine triphosphate. The triphosphate drug is also incorporated in to viral DNA where it acts as a chain terminator, because it has 3' - hydroxy group, like cyclic sugar, 3' 5' - phosphodiester bond can be formed. It acts as suicide inhibitor because the terminated DNA template containing acyclovir as ligand binds irreversibly with DNA polymerase and inactivates. It is a drug of choice in both prophylaxis and treatment of herpes simplex virus.

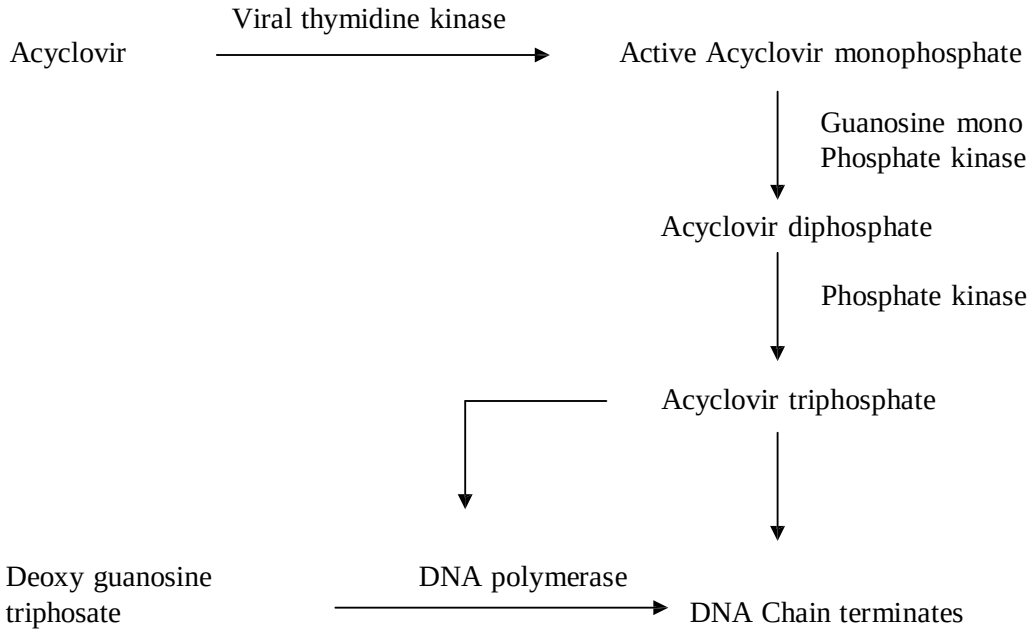


Fig. 2.1: Mechanism of Acyclovir

E. Pharmacokinetics:

- **Oral bioavailability** **:** 15-30%
- **Protein binding** **:** 9-33%
- **Volume of distribution** **:** 0.8 L/kg.
- **Half life** **:** 2.5-3.3 hours.

™ **Route of administration** **:** Especially oral, IV, cream 5% in an aqueous cream base and eye ointment.

F. Absorption:

Acyclovir is poorly absorbed from the gastro intestinal tract following oral administration. The oral bioavailability ranges from 10 to 20% and decreases with increased doses. The C_{max} and AUC are dose dependent and show no proportionality. The C_{max} for a bioavailability ranges from 10 to 20% and decreases with increased doses. The C_{max} and AUC are dose dependent and show no proportionality. The C_{max} for a 200 mg dose is 0.83 µg/mL, 1.21 µg/mL for a dose of 400 mg and 1.61 µg/mL for a dose of 800 mg. Oral absorption is not affected by food consumption. There is minimal systemic absorption following topical administration of acyclovir. No drug is detectable in the blood or urine. Following intravenous administration of acyclovir, the mean C_{max} is 9.8 µg/mL with a dose of 5 mg/kg every 8 hours and 20.7 µg/mL with a dose of 10 mg/kg every 8 hours.

G. Distribution:

The volume of distribution is 0.8 L/kg. Protein binding of acyclovir ranges from 9% to 33%, and distributes extensively throughout the body. The highest concentrations are in the kidneys, liver and intestines. Cerebro spinal fluid concentrations are about 50% that of the plasma. Acyclovir does cross the placenta.

H. Metabolism:

Acyclovir is converted to acyclovir monophosphate by virus specific thymidine kinase, diphosphate by cellular guanylate kinase, and then ultimately converted to acyclovir triphosphate via cellular enzymes. Acyclovir undergoes

minimal hepatic metabolism by aldehyde oxidase, alcohol dehydrogenase, and aldehyde dehydrogenase to form inactive metabolites.

I. Excretion:

Acyclovir is primarily eliminated by the kidneys via glomerular filtration and tubular secretion. It is found in the urine from 62% to 90% as unchanged drug and metabolites. The plasma elimination half life of Acyclovir ranges from 2.5 to 3.3 hours in patients with normal renal function.

J. Contraindications:

Hypersensitivity to valcyclovir, acyclovir, or any component of the formulations.

K. Precautions:

- ¾ Dosage reduction is recommended when administering acyclovir to patients with renal impairment.
- ¾ Use with caution in the elderly due to decreased renal function.
- ¾ Use with caution in patients who receive nephrotoxic drugs.
- ¾ Use caution when administering via IV in those with pre-existing neurologic abnormalities, serious hepatic or electrolyte abnormalities, or hypoxia.

L. Interactions:

- ¾ Probenecid: concomitant administration of probenecid and Cimetidine with acyclovir increases the acyclovir mean half life and under the plasma concentration curve, thus decreasing renal clearances of probenecid.

- ¾ Observe caution if administration nephrotoxic drugs simultaneous.
- ¾ Zidovudine: Acyclovir along with zidovudine causes sever drowsiness and lethargy.

M. Dosage forms:

- ¾ Intravenous infusion
- ¾ Capsule
- ¾ Tablet
- ¾ Suspension
- ¾ Topical cream
- ¾ Topical ointment

N. Dosage limits:

Capsules	**:** 200 mg
Tablets	**:** 400 mg, 800 mg
Suspension	**:** 200 mg/5mL
Powder for injection	**:** 500 mg, 1000 mg
Solution for injection	**:** 50 mg/5mL
Creams	**:** 5%
Ointment	**:** 5%

O. Over dosage Measures:

Precipitation of acyclovir in renal tubules may occur when the solubility (2.5 mg/mL) is exceeded in the intra tubular fluid. This can be treated with hemo dialysis until renal function is restored.

P. Commercial products of Acyclovir:

Table 2.1: Commercial products of Acyclovir

Trade name	**Preparation**	**Dose**	**Manufacturer**
Acivir	Tablet Injection Cream (5 %) Ointment (3 %)	200, 400 and 800 mg 10 mL 5 g 5 g	Cipla
Acivirall	DT- tablet	200 and 400 mg	Finecure
Alovir	Tablet	200, 400 and 800 mg	Adley
Axovir	Tablet Injection	200, 400 and 800 mg 250 and 800 mg vials	Samarth
Clovirax	DT- tablet Cream (5 %)	200, 400 and 800 mg 5 g	Purehealth
Cyclovir	Tablet Cream (5 %)	200 mg 5 g	Zydus cadilla
Herpex	DT- tablet Cream (5 %)	200 and 800 mg 5 g	Torrent
Lovir	Tablet	400 and 800 mg	Eli lilly
Ocuvir	Ointment (3 %)	5 g	FDC
Zovirax	Suspension Ointment (3 %) Tablet	400 mg/5 mL 5 g 200, 400 and 800 mg	GSK

2.3. POLYMER PROFILE

EUDRAGIT ®- EPO:

™ **Non-proprietary names:**

Ph. Eur: Basic Butylated Methacrylate Copolymer

USP/NF: polymer conforms to Amino Methacrylate Copolymer - NF

JPE: Aminoalkyl Methacrylate Copolymer E

™ **Synonyms:**

Eudragit®-EPO is a cationic copolymer based on dimethylaminoethyl methacrylate, butyl methacrylate, methyl methacrylate and acrylates/ dimethylaminoethyl methacrylate copolymer.

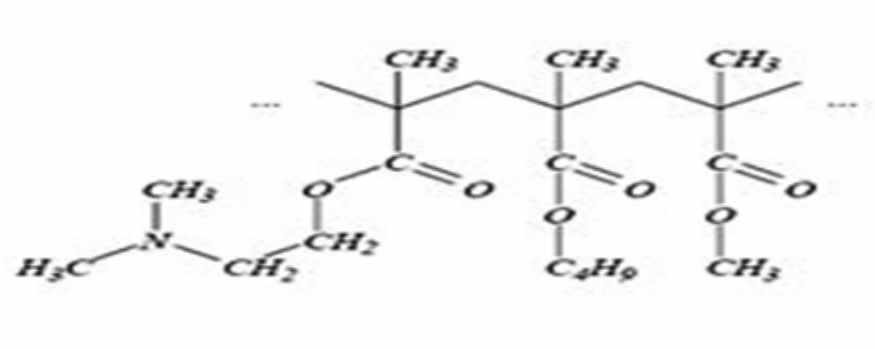
™ **Chemical/IUPAC name:**

Poly(butyl methacylate-co-(2-dimethylaminoethyl) methacrylate-co-methyl methacrylate) 1:2:1

™ **Molecular weight:**

approximately 47,000 g/mol

™ **Structural formula:**



™ **Functional catrgory:**

Film former; tablet binder; tablet diluents.

™ **Application in pharmaceutical formulation or technology:**

Eudragit®-EPO is used as a plain or insulating film former; it is soluble in gastric fluid below pH 5. In contrast, Eudragit L, S and FS types are used as enteric coating agents because they are resistant to gastric fluid. Different types are available that are soluble at different pH values: e.g. Eudragit L is soluble at pH > 6; Eudragit S and FS are soluble at pH > 7.

™ **Description:**

It is a white powder with a characteristic amine-like odour.

™ **Typical property:**

Alkili value **:** 162-192

Density (bulk) **:** 0.390 g/cm^3

Density (tapped) **:** 0.424g/cm^3

™ **Flash point** **:** not flammable

™ **Melting point** **:** 125°C

™ **Solubility:**

Eudragit®-EPO dissolves in 7 g methanol, ethanol, and isopropyl alcohol, acetone, ethyl acetate, methylene chloride or 1 N hydrochloric acid to give clear to slightlycloudy solutions.

™ **Stability and storage condition:**

Store at temperature up to 25°C (77 °F). Protect against moisture. PP box with Polyethylene inner bag.

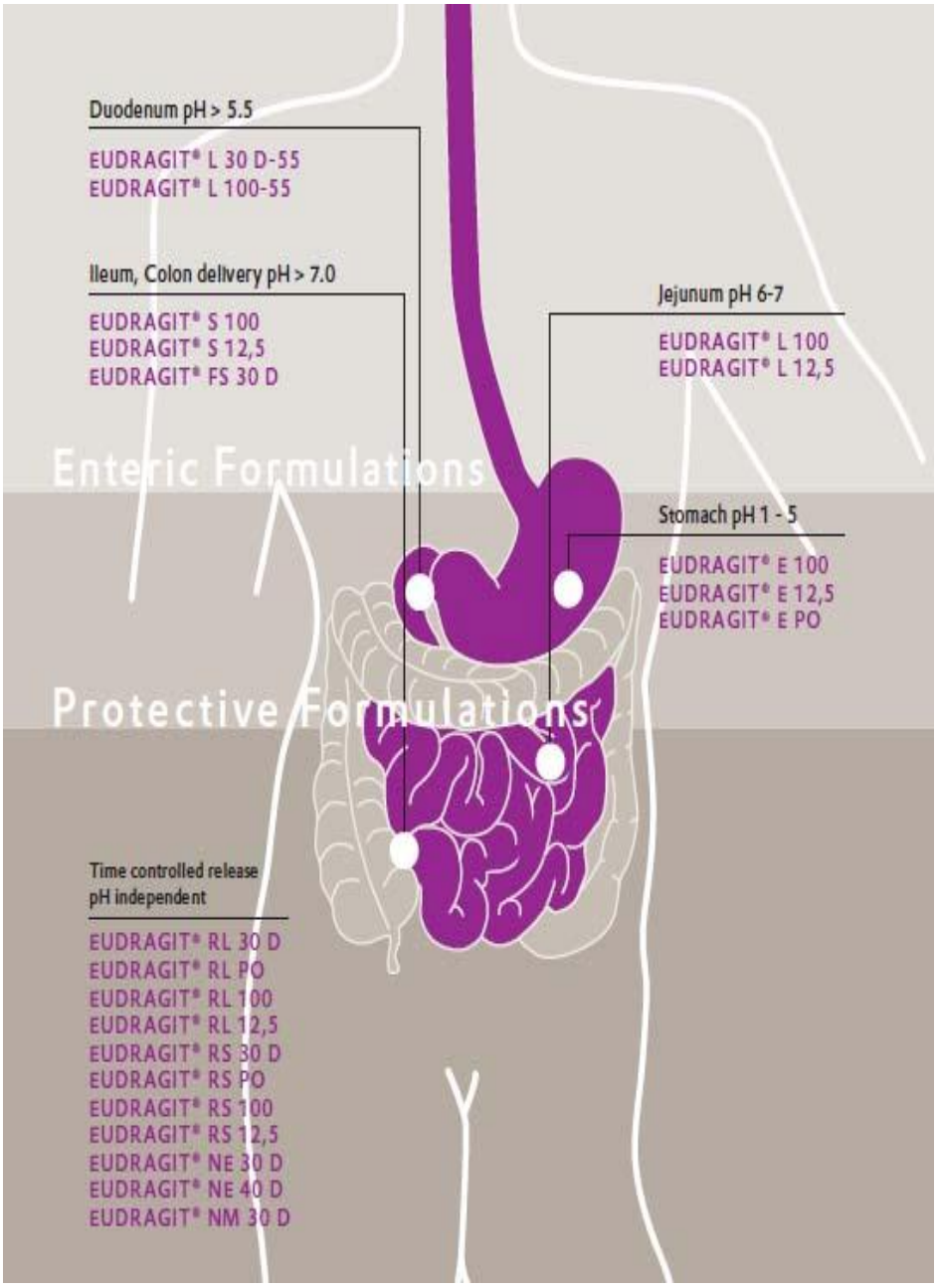


Fig. 2.2: pH sensitivity of the different grade Eudragit polymers

2.4. EXCIPENT PROFILE

PLURONIC ® F-68: *(Raymond C Rowe., 2003)*

™ **Nonproprietary names:**

BP : Poloxamers

PhEur : Poloxamera

USPNF: Poloxamer

™ **Synonyms:**

Lutrol; Monolan; Pluronic; poloxalkol; polyethylene–propyleneglycol copolymer; polyoxyethylene polyoxypropylene copolymer; Supronic; Synperonic.

™ **Chemical name and CAS registry number:**

a-Hydro-o hydroxypoly(oxyethylene)poly(oxypropylene) poly(oxyethylene) block copolymer.

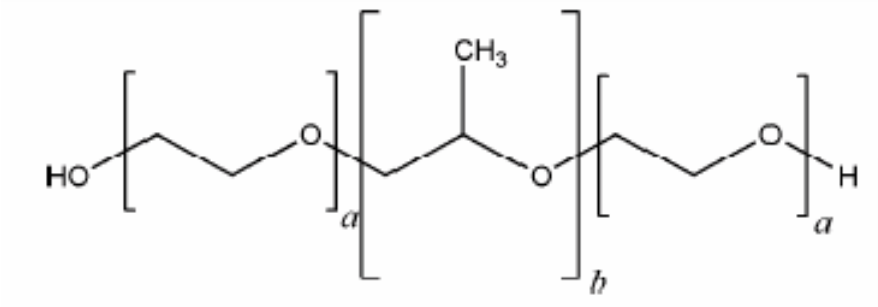
™ **Empirical formula:**

The Pluronic polyols are a series of closely related block copolymers of ethylene oxide and propylene oxide conforming to the general formula HO(C2H4O)a(C3H6O)b(C2H4O)aH.

™ **Molecular weight:**

Solid 80 27 7 680–9 510

™ **Structural formula:**



™ **Functional category:**

Dispersing agent

Emulsifying agent

Coemulsifying agent

Solubilising agent

Tablet lubricant

Wetting agent

™ **Applications in pharmaceutical formulation or technology:**

Pluronics are nonionic polyoxyethylene–polyoxypropylene copolymers used primarily in pharmaceutical formulations as emulsifying or solubilizing agents. The polyoxyethylene segment is hydrophilic while the polyoxypropylene segment is hydrophobic. All of the Pluronics are chemically similar in composition, differing only in the relative amounts of propylene and ethylene oxides added during manufacture. Their physical and surface-active properties vary over a wide range and a number of different types are commercially available. Pluronics are used as emulsifying agents in intravenous fat emulsions, and as solubilizing and stabilizing agents to maintain the clarity of elixirs and syrups. Pluronics may also be used as wetting agents, in ointments, suppository bases, and gels, as tablet binders and

coatings. Pluronic F-68 has also been used as an emulsifying agent for fluorocarbons used as artificial blood substitutes and in the preparation of solid-dispersion systems. More recently, Pluronics have found use in drug-delivery systems. Therapeutically, Pluronic F-6 is administered orally as a wetting agent and stool lubricant in the treatment of constipation; it is usually used in combination with a laxative such as danthron. Pluronics may also be used therapeutically as wetting agents in eye-drop formulations, in the treatment of kidney stones, and as skin-wound cleansers. Pluronic 338 and 407 are used in solutions for contact lens care.

Table 2.2: Applications of pluronic in pharmaceutical formulation

S.NO.	USE	CONCENTRATION
1	For emulsifier	0.3
2	Flavour solubilizer	0.3
3	Fluorocarbon emulsifier	2.5
4	Gelling agent	15-50
5	Spreading agent	1
6	Stabilizing agent	1-5
7	Suppository base	4-6 or 90
8	Tablet coating	10
9	Tablet excipient	5-10
10	Wetting agent	0.01-5

™ **Descriptions:**

Pluronics generally occur as white, waxy, free-flowing prilled granules, or as cast solids. They are practically odorless and tasteless. At room temperature, Pluronic 124 occurs as a colorless liquid.

™ **Typical properties:**

Acidity/alkalinity **:** pH = 5.0-7.4 for a 2.5% w/v aqueous solution.

Cloud poin **:** >1008C for a 1% w/v aqueous solution, and a 10% w/v Aqueous solution of Pluronic F-68 .

Density **:** 1.06 g / cm3 at 258ºC. **Flowability** **:** solid Pluronics are free flowing. **HLB value** **:** 0.5-30; 29 for Pluronic F-68 . **Melting point** **:** 52–578ºC for Pluronic F-68 .

™ **Moisture content:**

Pluronics generally contain less than 0.5% w/w water and are hygroscopic only at relative humidity greater than 80%.

™ **Solubility:** solubility varies according to the Pluronic type.

Freely soluable in ethanol,

Freely soluable in water.

™ **Surface tension:**

19.8mN/m (19.8 dynes/cm) for a 0.1% w/v aqueous Pluronic F-68 solution at 258ºC.

™ **Viscosity (dynamic):**

1000 mPa s (1000 cP) as a melt at 778C for Pluronic F-68.

™ **Stability and storage:**

Conditions Pluronics are stable materials. Aqueous solutions are stable in the presence of acids, alkalis, and metal ions. However, aqueous solutions support mold growth. The bulk material should be stored in a well-closed container in a cool, dry place.

™ **Incompatibilities:**

Depending on the relative concentrations, Pluronic F-68 is incompatible with phenols and parabens.

™ **Method of manufacture:**

Pluronic polymers are prepared by reacting propylene oxide with propylene glycol to form polyoxypropylene glycol. Ethylene oxide is then added to form the block copolymer.

™ **Safeties:**

Pluronics are used in a variety of oral, parenteral, and topical pharmaceutical formulations and are generally regarded as nontoxic and nonirritant materials. Pluronics are not metabolized in the body. Animal toxicity studies, with dogs and rabbits, have shown Pluronics to be nonirritating and nonsensitizing when applied in 5% w/v and 10% w/v concentration to the eyes, gums, and skin. In a 14-day study of intravenous administration at concentrations up to 0.5 g/kg/day to rabbits, no overt adverse effects were noted. A similar study with dogs also showed no adverse effects at dosage levels up to 0.5 g/kg/day. In a longerterm study, rats fed 3% w/w or 5% w/w of Pluronic in food for up to 2 years did not exhibit any significant symptoms of toxicity. However, rats receiving 7.5% w/w of Pluronic in their diet showed some decrease in growth rate. No hemolysis of human blood cells was observed over

18 hours at 258ºC, with 0.001–10% w/v Pluronic solutions. Acute animal toxicity data for Pluronic F-68.

™ **Handling precautions:**

Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection and gloves are recommended.

™ **Regulatory statuses:**

Included in the FDA Inactive Ingredients Guide (IV injections; inhalations, ophthalmic preparations; oral powders, solutions, suspensions, and syrups; topical preparations). Included in nonparenteral medicines licensed in the UK. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

AIM

AND

OBJECTIVE

3. AIM AND OBJECTIVE

Herpes simplex virus (HSV) is a member of family of herpes viridae, a DNA virus. There are two types of Herpes Simplex Viruses (HSV). viz HSV type 1 and type 2. HSV type 1 is the herpes virus that is usually responsible for cold sores of the mouth, the so called "fever blisters". HSV type 2 is the one that most commonly causes genital herpes. The infection causes painful sores on the genitals in both men and women. Herpes sores provide a way for HIV to get past the body's immune defenses and make it easier to get HIV infection. A recent study found that people with HSV had three times the risk of becoming infected with HIV as compared to people without HSV. Currently the treatments available for herpes simplex are conventional tablets and topical gel for application on outbreaks. The drugs that are commonly used for herpes simplex are Acyclovir, Valaclovir and Famciclovir.

Acyclovir, the first agent to be licensed for the treatment of herpes simplex virus infections, is the most widely used drug for infections such as cutaneous herpes, genital herpes, chicken pox, varicella zoster infections. Acyclovir is currently marketed as capsules (200 mg), tablets (200, 400 and 800 mg) and topical ointment. Oral acyclovir is mostly used as 200 mg tablets, five times a day. In addition, long term administration of acyclovir (6 month or longer) is required in immune compromised to patient with relapsing herpes simplex infection. The presently available conventional therapy is associated with a number of drawbacks such as highly variable absorption and low

bioavailability (10–20%) after oral administration. Furthermore, with increase in dose, there is decrease in bioavailability. Moreover, because the mean plasma half life of the drug is 2.5 hours, five times a day administration is required. In order to make oral therapy of acyclovir more patients compliant there is a need of using different approaches like matrix tablets, nanoparticle and polymeric films.

The main problem with the therapeutic effectiveness of acyclovir is its absorption which is highly variable and dose dependent thus reducing the bioavailability to 10–20%. Acyclovir is soluble in acidic pH and is predominantly absorbed from upper gastro intestinal tract (GIT) to duodenum to jejunum regions. There are indications of its active absorption from the duodenum and jejunum regions of GIT.

The inherent shortcomings of conventional drug delivery and the potential of nanoparticle as drug delivery systems have offered tremendous scope for researchers in this field and are fast moving from concept to reality. Nanoparticle may be used for oral administration of gut-labile drugs or those with low aqueous solubility. These colloidal carriers have the ability to cross the mucosal barrier as such. In addition to the potential for enhancing drug bioavailability via particle uptake mechanisms, nanoparticulate oral delivery systems also have slower transit times than larger dosage forms increasing the local concentration gradient across absorptive cells, thereby enhancing local and systemic delivery of both free and bound drugs across the gut. These colloidal carriers are expected to develop adhesive interactions within the mucosa and remain in the gastrointestinal tract, while protecting the entrapped drug from enzymatic degradation, until the release of the loaded drug or their absorption in an intact particulate form.

The purpose of this work was to develop Eudragit® EPO based nanoparticle suspension of acyclovir in order to increase its solubility and efficacy. The nanoparticle suspension was prepared by nanoprecipitation technique. The 3^2 factorial designs were used to study the effect of Eudragit® EPO and Pluronic® F-68 on characteristics of nanoparticle suspension of Acyclovir. The optimized formulation was subjected to lyophilization. The lyophilized nanoparticle suspension and the redispersed lyophilized nanoparticle suspension were characterized by particle size, drug content, entrapment efficiency, FTIR, DSC, SEM, in-*vitro* drug release, Kinetic drug release and stability studies.

PLAN OF WORK

4. PLAN OF WORK

- **LITERATURE SURVEY**
- **SELECTION OF DRUG, POLYMERS AND EXCIPIENTS**
- **PROCUREMENT OF DRUG, POLYMERS AND EXCIPIENTS**
- **EXPERIMENTAL WORK**
  - **Preformulation Study**
    - **Identification of Drug**
      - Organoleptic properties of drug
      - Melting point
      - Solubility profile
      - UV-Spectroscopy (λ_{max})
      - Quantification of drug
      - FTIR Spectroscopy
      - Loss on drying
  - **Formulation designing by 3^2 factorial designing technique**
  - **Formulation of pH sensitive nanoparticle**

- **Characterization of nanoparticle**
  - ¾ Particle size analysis
  - ¾ Estimation of drug content
  - ¾ Estimation of drug entrapment efficiency
  - ¾ Statistical analysis
  - ¾ Fourier transformer infrared spectroscopy (FTIR)
  - ¾ Differential scanning calorimetric analysis (DSC)
  - ¾ Scanning electron microscopy (SEM) analysis
  - ¾ Zeta potential measurement
  - ¾ *In-vitro* drug release study
  - ¾ Kinetics of *in-vitro* drug release
  - ¾ Stability study

RESULTS AND DISCUSSION

SUMMARY AND CONCLUSION

FUTURE PROSPECTS

BIBLIOGRAPHY

MATERIALS AND EQUIPMENT

5. MATERIALS AND EQUIPMENTS

5.1: Materials Used:

Table 5.1: List of Polymers and Excipients with source

S.No.	Ingredients	Supplier
1	Acyclovir	Ajanta pharmaceutical limited, Mumbai.
2	Eudragit® -EPO	Dr. Reddys pharmaceuticals limited, Mumbai.
3	Pluronic F-68	S d fine-chem limited, Mumbai.
4	Dialysis bag	Hi-media laboratories, Mumbai.
5	Hydrochloric acid	S d fine-chem. limited, Mumbai.
6	Methanol	Qualigens fine chemicals, Mumbai.
7	Ethanol (95%)	S d fine-chem limited, Mumbai.
8	Potassium dihydrogen orthophosphate	Fischer scientific chemicals, Mumbai.
9	Acetone	Fischer scientific chemicals, Mumbai.
10	Sodium hydroxide	Fischer scientific chemicals, Mumbai.

5.2: Equipments Used:

Table 5.2: List of Equipments with model/make

S.No	Equipments	Model/ Make
1	Electronic balance	Shimadzu BL-220H.
2	Sonicator	2200MH, Soltech srl, Soluzioni Tecnologirhe, Milano, Italy.
3	Magnetic stirrer	1-MLH, remi equipments limited, vasai.
4	Digital pH meter	Elico scientifics-L1610, Mumbai.
5	UV spectrophotometer	Shimadzu-1700 Pharmaspec UV-VISIBLE spectrophotometer.
6	FTIR spectrophotometer	Shimadzu S4008.
7	Differential scanning calorimeter	Shimadzu DSC 60 with DTA, Japan.
8	Micro ultra centrifuge	Remi equipments limited, vasai.
9	Freeze drying apparatus	Lyostar, S.P biotech limited.
11	Scanning electron microscope	SEM, JSM-6360LV scanning microscope Tokyo, Japan.
12	Zeta potential	Malveran instruments, Malvern, UK.
13	Humidity chamber	Labtech.

PREFORMULATION STUDIES

6. PREFPRMULATION STUDIES

Preformulation Study: *(Jens T., 2000)*

Before the formulation of a product should be investigation of physical and chemical properties of a drug substance alone to effective, stable and safe dosage form. It is the first step in rational development of dosage form.

6.1. Identification of Drug:

The preliminary studies were carried out by testing of different physical and chemical properties of drug as follows.

6.1.1. Organoleptic Properties of Drug: *(Lachman L., 1991)*

The Organoleptic properties like physical state, color, taste, odor etc., of the drug was reported with help of the descriptive terminology. It helps to identify the drug.

6.1.2. Melting Point: *(IP., 2007)*

It is the easy way to identify the drug. The melting point of acyclovir was tested by use of a laboratory melting point apparatus with capillary tube method a procedure given in the Indian Pharmacopeia 2007.

6.1.3. Solubility Profile: *(IP., 2007)*

It is important to know about solubility characteristic of a drug in aqueous system, since they must possess some limited aqueous solubility to elicit a therapeutic response. The solubility of drug was recorded by using various descriptive terminology specified in Indian pharmacopoeia, 2007.

Table 6.1: Description of solubility

Descriptive term	Parts of solvent required for 1 part of solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1,000
Very slightly soluble	From 1,000 to 10,000
Practically insoluble	Greater than or equal to 10,000

6.1.4. UV Spectroscopy (λ max): *(Kuchekar B. S., 2006)*

The absorption maximum of the standard solution was scanned between 200- 400 nm regions on Shimadzu-1700 Pharmaspec UV-visible spectrophotometer. The absorption maximum obtained with the substance being examined corresponds in position and relative intensity to those in the reference spectrum.

6.1.4.1. Development of Standard Curve of Acyclovir in Distilled Water:

(Kuchekar B. S., 2006)

Preparation of Stock Solution of Acyclovir in Distilled Water:

Weighed accurately about 100 mg of Acyclovir was dissolved in little quantity of distilled water and volume was adjusted to 100 ml with the same to prepare standard solution having concentration of 1000 µg/ml. From this solution, pipette out 10 ml and made up to 100 ml with distilled water to produce 100 µg/ml.

Procedure:

From the stock solution, aliquots of 0.4, 0.8, 1.2, 1.6 and 2 ml were transferred to 10 ml volumetric flasks and final volume was made to 10 ml with

distilled water to get 4 to 20 µg/ml. Absorbance values of these solutions were measured against blank (Distilled water) at 252 nm using UV-visible spectrophotometer.

6.1.4.2. Development of Standard Curve of Acyclovir in 0.1N HCl:

Preparation of 0.1N HCl:

0.1N HCl was prepared according to I.P. 1996. Accurately 8.5 ml of HCl was taken and diluted with freshly prepared distilled water to produce 1000 ml.

Preparation of Stock Solution of Acyclovir in 0.1N HCl:

Weighed accurately about 100 mg of Acyclovir was dissolved in little quantity of 0.1N HCl and volume was adjusted to 100 ml with the same to prepare standard solution having concentration of 1000 µg/ml. From this solution, pipette out 10 ml and made up to 100 ml with 0.1N HCl to produce 100 µg/ml.

Procedure:

From the stock solution, aliquots of 0.4, 0.8, 1.2, 1.6 and 2 ml were transferred to 10 ml volumetric flasks and final volume was made to 10 ml with 0.1N HCl to get 4 to 20 µg/ ml. Absorbance values of these solutions were measured against blank (0.1N HCl) at 255 nm using UV-visible spectrophotometer.

6.1.4.3. Development of Standard Curve of Acyclovir in pH 7.4 Phosphate Buffer:

Preparation of 0.2M Potassium Dihydrogen Phosphate:

Dissolved 27.218 gm of potassium dihydrogen phosphate in water and made up to 1000 ml.

Preparation of 0.2M Sodium Hydroxide:

Dissolved 8 gm of sodium hydroxide pellets in distilled water and made up to 1000 ml.

Preparation of Phosphate Buffer pH 7.4:

Phosphate buffer pH 7.4 was prepared according to I.P. 2007. A quantity of 50 ml of 0.2M potassium dihydrogen phosphate and 39.1 ml of 0.2M sodium hydroxide was diluted with freshly prepared distilled water to produce 200 ml.

Preparation of Stock Solution of Acyclovir in pH 7.4 Phosphate Buffer:

Weighed accurately about 100 mg of Acyclovir was dissolved in little quantity of pH 7.4 phosphate buffer and volume was adjusted to 100 ml with the same to prepare standard solution having concentration of 1000 µg/ml. From this solution, pipette out 10 ml and made up to 100 ml with pH 7.4 phosphate buffer to produce 100 µg/ml.

Procedure:

From the stock solution, aliquots of 0.4, 0.8, 1.2, 1.6 and 2 ml were transferred to 10 ml volumetric flasks and final volume was made to 10 ml with pH 7.4 phosphate buffer to get 4 to 20 µg/ml. Absorbance values of these solutions were measured against blank (Phosphate Buffer pH 7.4) at 253 nm using UV-visible spectrophotometer.

6.1.5. Quantification of Drug:

Accurately weighed 100 mg of Acyclovir was dissolved in little quantity of pH 7.4 phosphate buffer and volume was adjusted to 100 ml with the same to prepare standard solution having concentration of 1000 µg/ml. From this solution, pipette out 10 ml and made up to 100 ml with pH 7.4 phosphate buffer to produce 100 µg/ml.

From the above solution, aliquots of 2 ml were transferred to 10 ml volumetric flasks and final volume was made to 10 ml with pH 7.4 phosphate buffer. Absorbance values of these solutions were measured against blank (pH 7.4 Phosphate Buffer) at 253 nm using Shimadzu-1700 Pharmaspec UV-visible spectrophotometer. The percentage purity of drug was calculated by using calibration graph method (least square method).

6.1.6. FTIR Spectroscopy: *(selvaraj S., 2010)*

The infrared spectrum was generally used as an identification parameter to know the chemical structure of drugs. The FTIR spectrophotometer was used to record the FTIR spectrum of acyclovir. A small quantity of sample was mixed with sufficient potassium bromide and compressed into a pellet by applying a 10 tons pressure with help of a hand operated press. This pellet was kept in a sample holder and scanned from 4000 to 400 cm^{-1}

6.1.7. Loss on Drying: *(IP., 2007)*

Loss on drying is the loss of weight expressed as percentage w/w resulting from water and volatile matter of any kind that can be driven off under specified condition. The accurately weighed 1gm of sample was transferred in glass-stoppered, shallow weighing bottle and accurately weighed the bottle. The bottle was transferred in oven and substance was dried at 105°C for 3 hours. The bottle was removed from oven and reweighed; loss on drying was calculated by following equation,

$$\textbf{LOD} = \frac{\textbf{Initial weight of substance - Final weight of substance}}{\textbf{Initial weight of substance}} \times 100$$

FORMULATION OF pH SENSITIVE NANOPARTICLE

7. FORMULATION OF P^{H} SENSITIVE NANOPARTICLE

7.1. Formulation Designing by 3^2 Factorial Designing Technique:

(*Bothiraja C., 2009*)

A prior knowledge and understanding of the process and the variable under investigation led to preliminary experiments. Based on the preliminary data, the 3^2 factorial design was adopted to optimize the amount of Eudragit®-EPO (x_1) and pluronic® F-68 (x_2) identify the independent variable affecting the drug content and the percentage drug encapsulation efficiency (dependent variable). The response surfaces of the obtained result were also plotted. The coded and the actual values of the experimental design are given in Table 7.1. The data analysis of values obtained from various batches for drug content and entrapment efficiency were subjected to multiple regression analysis using PCP dissolution software the equation fitted was

$$Y: \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2$$

Where y is the measured response; X is the level of factors; β is the coefficient computed from the responses of the formulations.

Table 7.1: Independent variables and their selected levels for nanoparticles formulation

S. No.	Independent variable factor	Levels used		
		Lower (-1) (mg)	**Middle (0) (mg)**	**Upper (+1) (mg)**
1	Eudragit®-EPO	100	150	200
2	Pluronic F-68	25	50	100

Table 7.2: Formulation of Acyclovir loaded pH sensitive nanoparticle using 3^2 full factorial design

S.No	Batch code	Variable level in code form	
		X_1	**X_2**
1	F1	-1	-1
2	F2	-1	0
3	F3	-1	+1
4	F4	0	-1
5	F5	0	0
6	F6	0	+1
7	F7	+1	-1
8	F8	+1	0
9	F9	+1	+1

7.2: Preparation of pH Sensitive Nanoparticle Suspension:

(*Bothiraja C., 2009*; *Atmaram P. Pawer., 2011)*

Nanoparticle suspension of acyclovir was prepared by nanoprecipitation method. Acyclovir 100 mg and specific amount of Eudragit®-EPO were dissolved in 15 ml of methanol. The organic solution was quickly injected to 30 ml aqueous solution containing pluronic® F-68 under stirring at 2000 rpm. Stirring was continued for 2 hours at 40^0C for complete evaporation of methanol. The volume was adjusted up to 40 ml with aqueous solution of 200 mg of HPMC K-15 to obtain a nanoparticle suspension. The optimized nanoparticle suspension was lyophilized at – 42^0C for 72 hours and which was also redispersed in water to get aqueous nanoparticle suspension

Figure7.1: Preparation of pH sensitive nanoparticle

CHARACTERIZATION OF NANOPARTICLE

8. CHARACTERIZATION OF NANOPARTICLE

8.1. Particle Size Analysis: **(***Yadav A.V., 2009)*

Particle size analysis of nanoparticle was performed by photon correlation spectroscopy (PCS). This technique yields the mean particle diameter and particle size distribution. Samples were analyzed using Mastersizer 2000 (Malvern Instruments, Malvern, UK), which allows sample measurement the range of 0.020 – 2000.00 µm. Polydispersity was determined according to the equation:

$$\textbf{Polydispersity} = \frac{\textbf{D (0.9) – D (0.1)}}{\textbf{D (0.5)}}$$

Where D (0.9) corresponds to particle size immediately above 90% of the sample, D (0.5) corresponds to particle size immediately above 5% of the sample, D (0.1) corresponds to particle size immediately above 10% of the sample.

8.2. Estimation of Drug Content: ***(****Panchaxari M Dandagi., 2011)*

The nanoparticle suspension was weighed accurately (equivalent to 25 mg) of the acyclovir nanoparticle suspension was diluted with 0.1N HCl and the drug content were estimated by using UV -Visible spectrophotometer (Shimadzu UV-1700) at 253 nm against blank solvent system**.**

$$\textbf{Percentage drug content} = \frac{\textbf{Amount of drug found}}{\textbf{Label claim}} \times \textbf{100}$$

8.3. Estimation of Entrapment Efficiency: *(Selvaraj S., 2010)*

The entrapment efficiency of the prepared formulation was determined by the concentration of free drug in the dispersion medium. The entrapment drug was determined by taking 10 ml of formulation the nanoparticle suspension was centrifuged for 2 hours at 14000 rpm. The supernatant was separated and filtered through 0.45µm illipore (Millipore filter). The filtrate was diluted with 0.1N HCl and measured specrophotometrically (Shimadzu UV-1700) the amount of free drug was determined in filtered drug was determined. Initial drug –free drug the entrapment efficiency was calculated using the following equation.

$$\textbf{Percentage drug entrapment efficiency} = \frac{\textbf{W initial drug} - \textbf{W free drug}}{\textbf{W initial drug}} \times \textbf{100}$$

8.4. Statistical Analysis: (*Bothiraja C., 2009*; *Atmaram P. Pawer., 2011)*

A prior knowledge and understanding of the process and the variable under investigation led to preliminary experiments. Based on the preliminary data, the 3^2 factorial design was adopted to optimize the amount of Eudragit®-EPO (x_1) and Pluronic® F-68 (x_2) identify the independent variable affecting the drug content and the percentage drug encapsulation efficiency (dependent variable). The response surfaces of the obtained result were also plotted. The data analysis of values obtained from various batches for drug content and encapsulation efficiency were subjected to multiple regression analysis using PCP dissolution software the equation fitted was

$$Y: \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad ....1$$

Where y is the measured response; X is the level of factors; β is the coefficient computed from the responses of the formulations.

8.5. Fourier Transform Infra Red Spectroscopy (FTIR): *(Selvaraj S., 2010)*

The FTIR spectra of Acyclovir, Eudragit®-EPO and Acyclovir loaded Eudragit®-EPO nanoparticles were determined by using Shimadzu S4008 model. The pellets were prepared by gently mixing of 1mg sample with 200 mg potassium bromide at high compaction pressure. A base line correction was made using dried potassium bromide and the spectra of dried mixture of drug and polymers were recorded. Thus the prepared pellet was scanned at a resolution of from 4000 cm^{-1} to 400 cm^{-1}.

8.6. Differential Scanning Calorimetry Analysis (DSC): *(Vikram M Pandya., 2011)*

Differential scanning calorimetry (DSC) are one of the most powerful analysis technique which offering the possible of detecting chemical interaction between drug and polymer. The differential scanning calorimetry of thermograms of Acyclovir, Eudragit®-EPO, and lyophilized nanoparticle suspension were obtained using DSC-Shimadzu 60 with TDA trend line software.

8.7. Scanning Electron Microscopy (SEM) Analysis: *(Anilkumar J., 2011)*

The morphology of nanoparticle was examined by using scanning electron microscopy (SEM, JSM-6360LV scanning microscope Tokyo, Japan). The nanoparticle formulation was mounted on metal stubs using double-sided tape and coated with a 150 A^0 layer of gold under vacuum. Stubs were visualized under scanning electron microscope. SEM has been used to determine particle size distribution, surface topography, texture and examine the morphology of fractured or sectioned surface.

8.8. Zeta Potential Measurement: (*Vikas Jain., 2009*)

The zeta potential was determined by (Malvern Instruments, Malveran UK). It is a measure of electric charge at the surface of particles indicating the physical stability of dispersive systems. The zeta potential values higher than 30 mV indicate long term electrostatic stability of aqueous dispersions. The analysis was performed using the clear disposable zeta cell, in distilled water at 20 V/cm of field strength. All measurements were done in triplicate.

8.9. *In-Vitro* Drug Release Study: ***(****Panchaxari M Dandagi., 2011)*

The *in-vitro* drug release of the nanoparticle suspension was studies by using dialysis method the formulation equivalent to 10 mg of acyclovir were placed in a dialysis bag. The dialysis bag was suspended in a beaker containing 100 ml of phosphate buffer pH 7.4 on a magnetic stirrer at 100 rpm, with temperature adjusted to 37±0.5°C at selected time interval sample was removed and replaced with fresh medium. The sample was filtered through 0.45µm Millipore (Millipore filter) the samples were analyzed for drug release by measuring absorbance 252 nm using UV visible spectrophotometer (Shimadzu UV-1700).

Figure 8.1: *In-vitro* drug release of pH sensitive nanoparticle

8.10. Kinetics of *In-Vitro* Drug Release: *(Amitava G., 2009)*

¾ **Zero order:**

$$C = K_0 t$$

Where K_0 - is the zero-order rate constant expressed in units of concentration/time

t -is the time in h.

¾ **First order:**

$$LogC = LogC_0 - Kt / 2.303$$

Where C_0 - is the initial concentration of drug,

K - is the first order constant

t - is the time in h.

¾ **Higuchi:**

$$Q_t = Kt^{1/2}$$

Where Q_t - is the amount of the release drug in time t,

K- is the kinetic constant and t- is time in h.

¾ **Korsmeyer Peppas:**

$$M_t / M_\infty = Kt^n$$

Where M_t - represents amount of the released drug at time t,

M_∞- is the overall amount of the drug (whole dose) released after 12 h

K- is the diffusional characteristic of drug/polymer system constant

n- is a diffusional exponent that characterizes the mechanism of release of drug.

Table 8.1: Diffusion exponent and solute release mechanism

Diffusion exponent (n)	**Overall solute diffusion mechanism**
< 0.5	Quasi-Fickian diffusion
0.5	Fickian diffusion
0.5 < n < 1.0	Anomalous (non-Fickian) diffusion
1.0	Case-II transport
> 1.0	Super case-II transport

8.11. Stability Study:

(Guru Prasad Mohanda., 2006; Rosario Pingnatello., 2006; IP., 2007)

A pharmaceutical product needs to be physically, chemically, therapeutically toxicologically and microbiologically stable throughout its shelf life. The pharmaceutical companies do stability testing for estimating the shelf life and based on this the expiry data is given for the product.

The real time studies (Long term testing) at recommended storage condition are ideal method for predicting shelf life. Often the studies are designed to increase the rate of chemical degradation or physical change of pharmaceutical products by

using exaggerated storage conditions. This is known as accelerated stability testing. The pharmaceutical products are subjected to higher temperature and humidity conditions for accelerating the degradation. However, the results of accelerated testing are not always predictive of physical changes and potency.

The pharmacopeia specifies certain storage conditions. The following table gives the details as specified in Indian pharmacopeia:

Table 8.2: Storage condition of pharmaceutical product

Storage condition	Meaning
Cold	Any temperature not exceeding 8ºC (2-8ºC)
Cool	Any temperature not between 8-25ºC
Warm	Any temperature not between 30-40ºC
Excessive heat	Any temperature not above 40ºC)

¾ **Procedure:**

The physical stability of the nanoparticle formulation F6 was evaluated after storage for 3 months at 4ºC ±1ºC and 25ºC ±2ºC/65% ±5% relative humidity. The nanoparticle were stored in screw capped amber-glass bottles and placed in a humidity control oven. Physical instability like change in appearance, settling behavior was observed. The samples were withdrawn and analyzed for its drug content, drug entrapment efficiency and *in-vitro* drug release.

RESULTS AND DISCUSSION

9. RESULTS AND DISCUSSION

9.1. Identification of Drug:

9.1.1. Organoleptic Properties:

Physical state : Fine powder

Colour : A white fine powder

Odour : Characteristic

Taste : Bitter to alkaline

9.1.2. Melting Point:

Melting point of Acyclovir was found to be 256.6 $\pm 1.15^{0}$C with decomposition. The official melting point range for Acyclovir is between 256-258°C. Hence, results were complied the limits specified in official Book.

9.1.3. Solubility Profile:

Table 9.1: Solubility of Acyclovir in various solvents

Name of solvent	**Parts of solvent required per part of solute**	**Solubility**
Dilute HCl	11	Soluble
Water	145	Slightly soluble
Alcohol	1000	Sparingly soluble
Acetone	More than 1000	Insoluble

The results were complied with official solubility of Acyclovir (IP 2007)

9.1.4. UV Spectroscopy (λ_{max}):

9.1.4.1. Determination of λ_{max} of Acyclovir by using Distilled Water:

The absorption maximum for Acyclovir in Distilled Water was found to be 252 nm and it is shown in Figure 9.1.

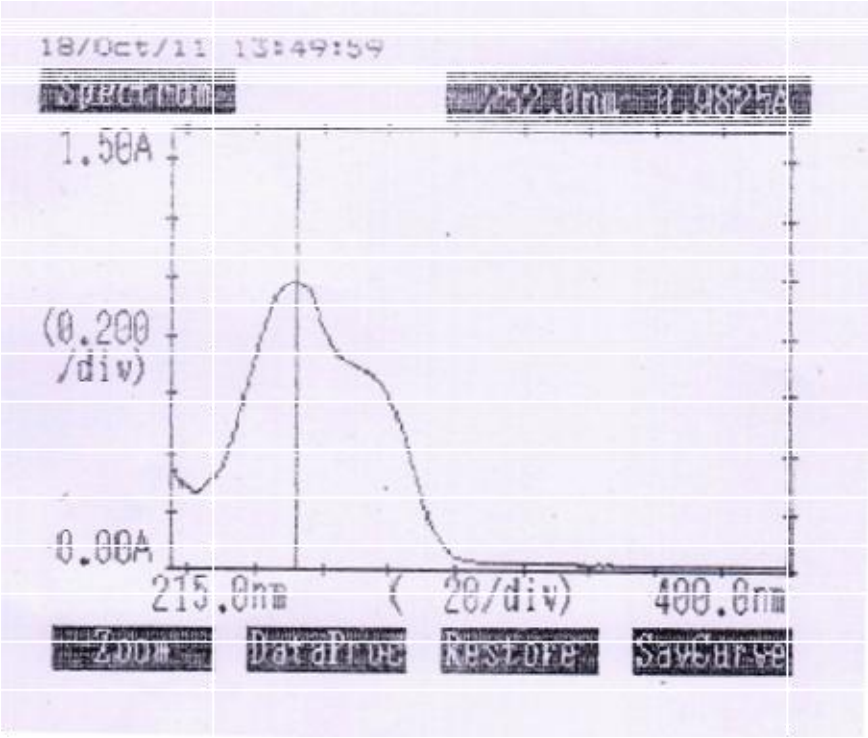


Fig. 9.1: λ_{max} Observed for Acyclovir in Distilled Water

9.1.4.2. Preparation of Standard Curve of Acyclovir by using Distilled Water:

UV absorption spectrum of Acyclovir in Distilled Water showed λ_{max} at 252 nm. Absorbance obtained for various concentrations of Acyclovir in distilled water are given in Table 9.2. The graph of absorbance vs. concentration for Acyclovir was found to be linear in the concentration range of 4 - 20 µg/ml. The drug obeys Beer - Lambert's law in the range of 4 - 20 µg/ml which is shown in Figure 9.2. The calibration parameters were shown in Table 9.3.

Table 9.2: Data of concentration and absorbance for Acyclovir for Distilled Water

S. No.	Concentration (µg/ml)	Absorbance at 252nm
1.	0	0
2.	4	0.191
3.	8	0.375
4.	12	0.563
5.	16	0.744
6.	20	0.931

Table 9.3: Data for calibration curve parameters in Distilled Water

S. No.	Parameters	Values
1.	Correlation Coefficient (r)	0.9999
2.	Slope (m)	0.04644
3.	Intercept (c)	0.00290

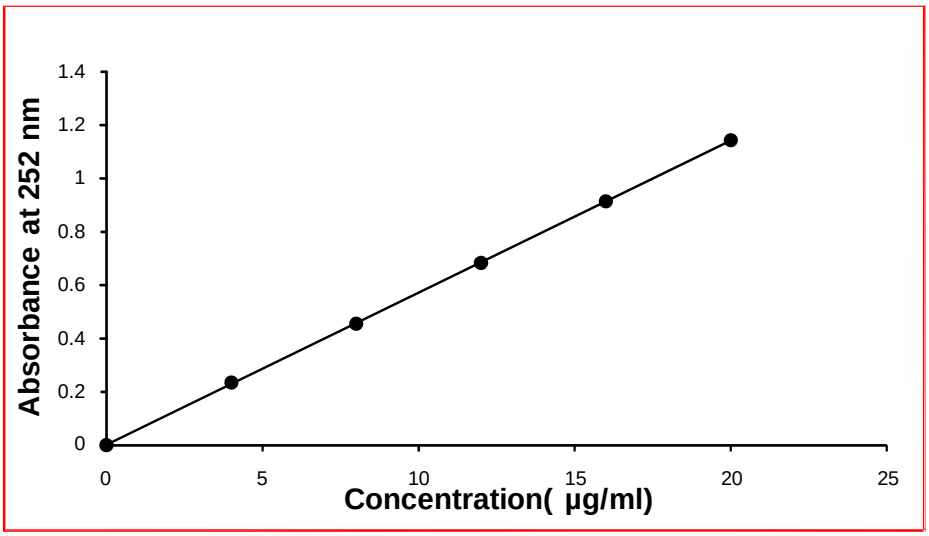


Fig. 9.2: Standard graph of Acyclovir in Distilled Water

9.1.4.3. Determination of λ_{max} of Acyclovir by using 0.1N HCl:

The absorption maximum for Acyclovir in 0.1N HCl was found to be 255.5 nm and it is shown in Figure 9.3.

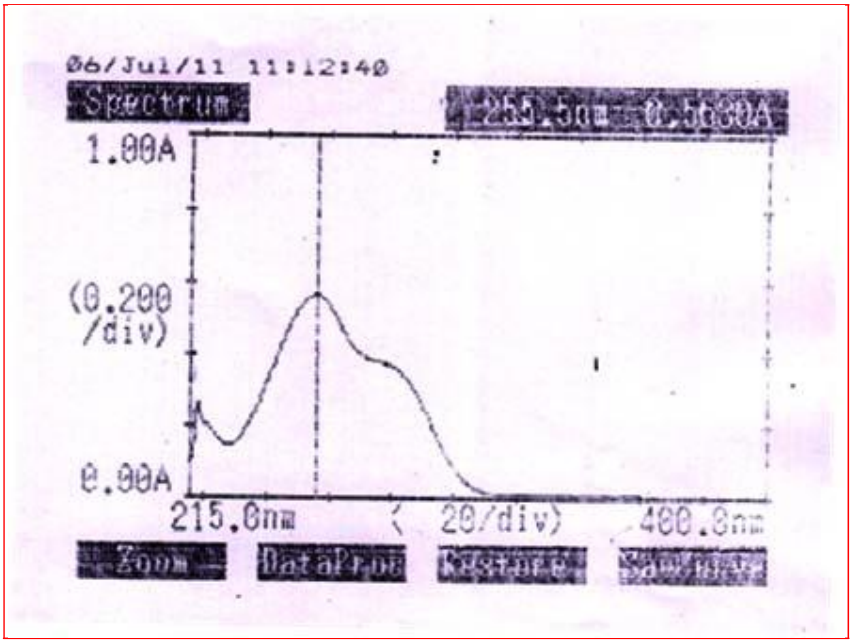


Fig. 9.3: λ_{max} Observed for Acyclovir in 0.1N HCl

9.1.4.4. Preparation of Standard Curve of Acyclovir by using 0.1N HCl:

The UV absorption spectrum of Acyclovir in 0.1N HCl shown λ_{max} at 255.5nm. Absorbance obtained for various concentrations of Acyclovir in 0.1N HCl are given in Table 9.4. The graph of absorbance vs. concentration for Acyclovir was found to be linear in the concentration range of 4 - 20µg /ml. The drug obeys Beer - Lambert's law in the range of 4 - 20µg /ml which is shown in Figure 9.4. The calibration parameters were shown in Table 9.5.

Table 9.4: Data of concentration and absorbance for Acyclovir for 0.1N HCl

S. No.	Concentration (µg/ml)	Absorbance at 255.5 nm
1.	0	0.000
2.	4	0.235
3.	8	0.455
4.	12	0.683
5.	16	0.914
6.	20	1.143

Table 9.5: Data for calibration curve parameters in 0.1N HCl

S. No.	Parameters	Values
1.	Correlation Coefficient (r)	0.9999
2.	Slope (m)	0.057
3.	Intercept (c)	0.0016

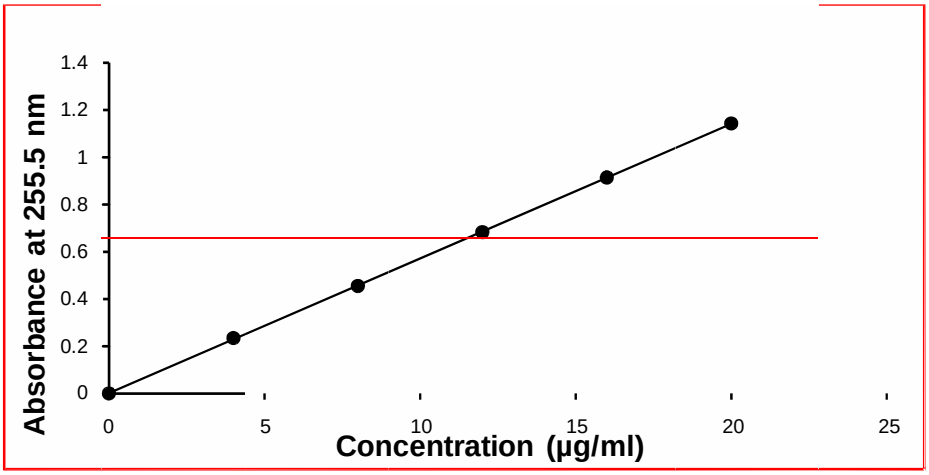


Fig. 9.4: Standard graph of Acyclovir in 0.1N HCl

9.1.4.5. Determination of λ_{max} of Acyclovir by using pH 7.4 Phosphate Buffer:

The absorption maximum for Acyclovir in pH 7.4 Phosphate Buffer was found to be 253 nm and it is shown in Figure 9.5.

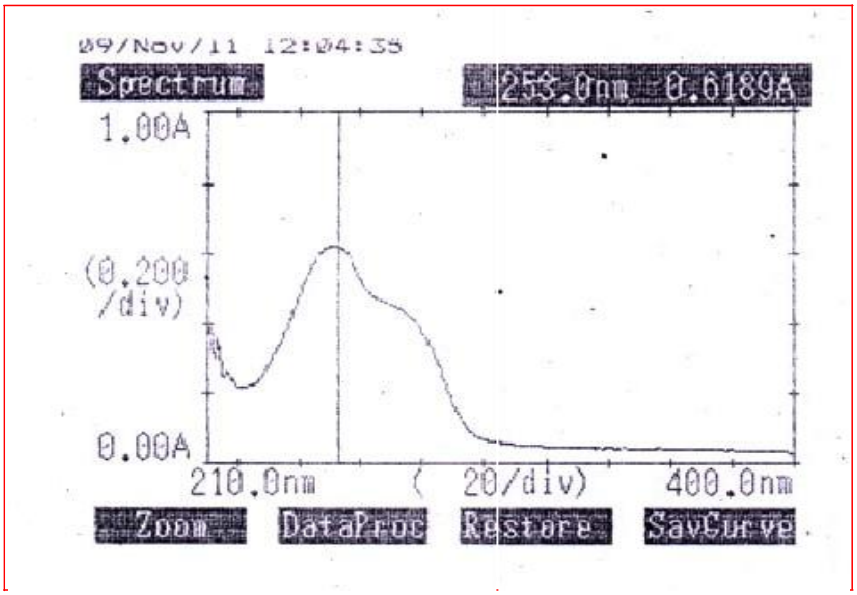


Fig. 9.5: λ_{max} Observed for Acyclovir in pH 7.4 Phosphate Buffer

9.1.4.6. Preparation of Standard Curve of Acyclovir by using pH 7.4 Phosphate Buffer:

UV absorption spectrum of Acyclovir in pH 7.4 Phosphate Buffer shown λ_{max} at 253 nm. Absorbance obtained for various concentrations of Acyclovir in pH 7.4 Phosphate Buffer are given in Table 9.6. The graph of absorbance vs. concentration for Acyclovir was found to be linear in the concentration range of 4 - 20µg/ml. The drug obeys Beer- Lambert's law in the range of 4 - 20µg/ml which is shown in Figure 9.6. The calibration parameters were shown in Table 9.7.

Table 9.6: Data of concentration and absorbance for Acyclovir in pH 7.4 Phosphate Buffer

S.No.	Concentration (µg/ml)	Absorbance at 253 nm
1.	0	0.000
2.	4	0.247
3.	8	0.494
4.	12	0.736
5.	16	0.982
6.	20	1.227

Table 9.7: Data for calibration curve parameters in pH 7.4 Phosphate Buffer

S. No.	Parameters	Values
1.	Correlation Coefficient (r)	0.9998
2.	Slope (m)	0.061331
3.	Intercept (c)	0.001333

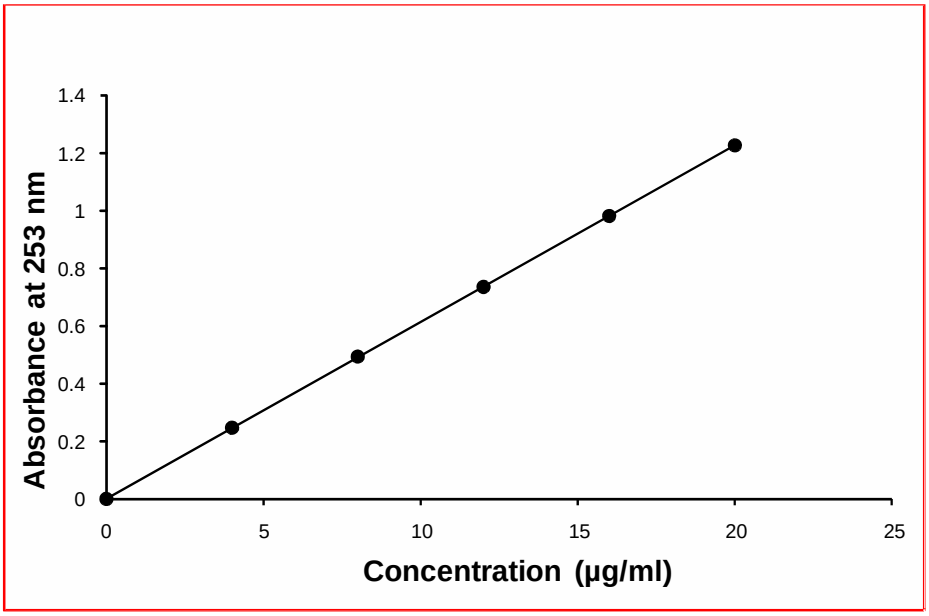


Fig. 9.6: Standard graph of Acyclovir in pH 7.4 Phosphate Buffer

9.1.5. Quantification of Drug:

The percentage purity of drug was calculated by using calibration graph method (least square method) and the data has been shown in Table 9.8.

Table 9.8: Percentage purity of Acyclovir

S. No.	Percentage Purity (%)	Average Percentage Purity* (%)
1.	101.38	101.03 ± 0.40
2.	100.57	
3.	101.06	

*All the values are expressed as mean ± SD, n = 3

The percentage purity for Acyclovir in IP 2007 is not less than 98.0 % and not more than 102.0 % of the stated amount of Acyclovir. The percentage purity of Acyclovir was found to be 101.03 ± 0.40. So, it stands within the limits of IP 2007.

9.1.6. FTIR Spectroscopy:

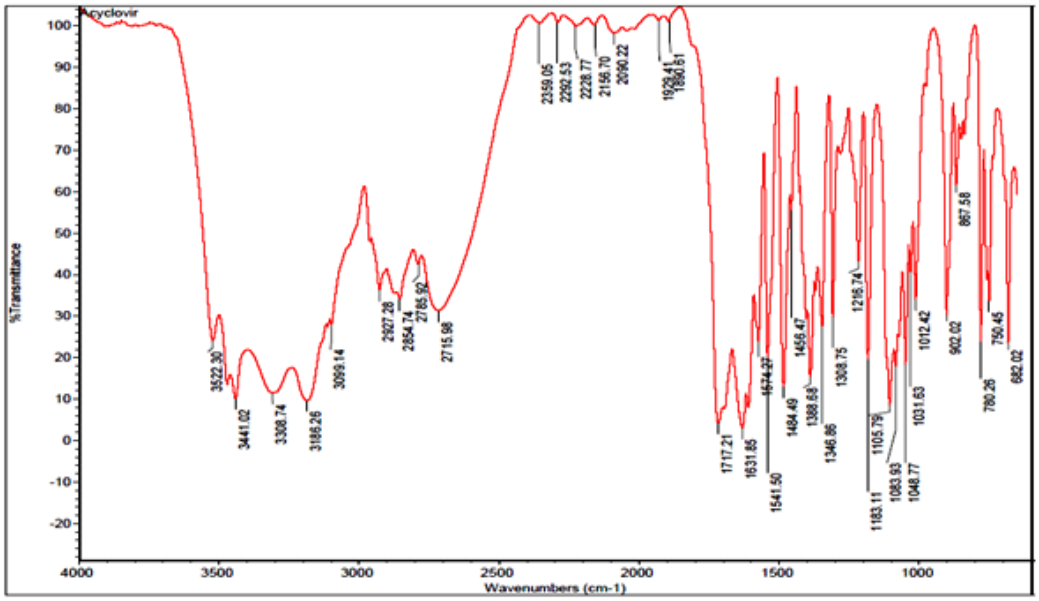


Fig. 9.7: FTIR spectroscopy of pure drug Acyclovir

Table 9.9: Characteristic frequencies in FTIR spectrum of Acyclovir

S.No.	Wave number (cm^{-1})	Inference
1	3441.02	N-H stretching
2	3308.74	O-H stretching
3	3099.14	C-H stretching
4	1717.21	C=O stretching
5	1541.50	C=C and C=N stretching
6	1308.75	$-CH_2$ Wagging and twisting
7	1216.74	Aryl alkyl ether
8	1105.79	C-O stretching
9	1083.93	C-O-C stretching

The drug was confirmed as Acyclovir with results obtained from FTIR spectrum analysis.

9.1.7. Loss on Drying:

The percentage loss on drying after 3 hours was found to be as follows

Table 9.10: Percentage loss on drying for Acyclovir

S. No	Percentage LOD	*Avg. percentage LOD
1	0.328	0.407±0.070
2	0.431	
3	0.463	

*All values are expressed as mean± S.D., n=3

The sample passes test for loss on drying as per the limit specified in IP, 2007 (N.M.T. 1%)

9.2. Characterization of Nanoparticle:

9.2.1. Particle Size Analysis:

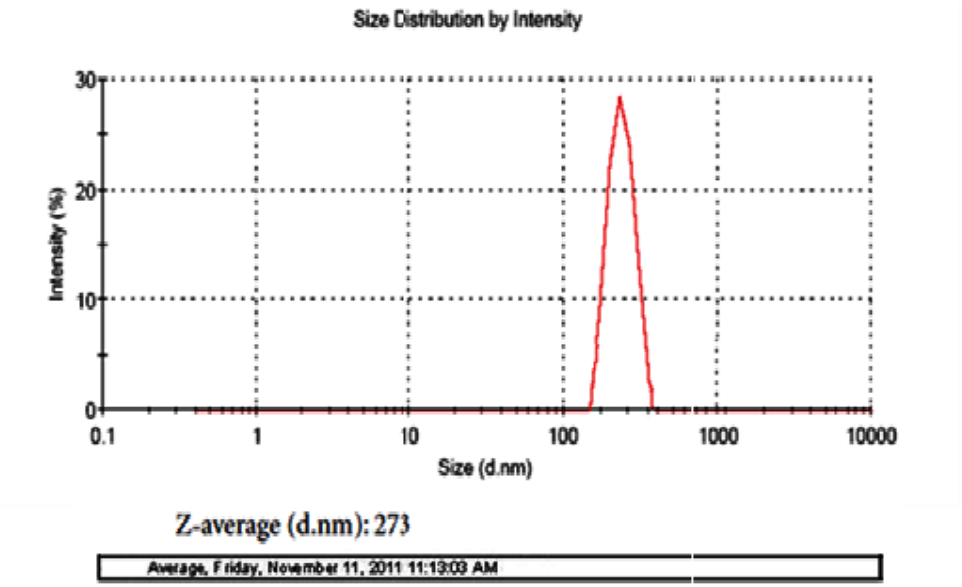


Fig. 9.8: Particle size graph of Acyclovir loaded pH sensitive nanoparticle formulation F6

Particle size of the nanoparticle suspension was in the range from 273 to 365 nm. The mean particle size of the nanoparticle suspension 271 ±3.83 and poly dispersity index 0.734 ±0.36. Increase in particle size of nanoparticle suspension with decrease in the polydispersity index was observed with increase in the polymer content. The smaller particle size obtained at low polymer content may be due to high distribution efficiency of the internal polymer-solvent phase into the external phase. Increase in the viscosity of internal phase with increased amount of polymer also provides resistance for mass transfer in turn diffusion of polymer-solvent phase into the external phase leading to particle enlargement. The results are shown in Figure 9.8.

9.2.2. Estimation of Drug Content:

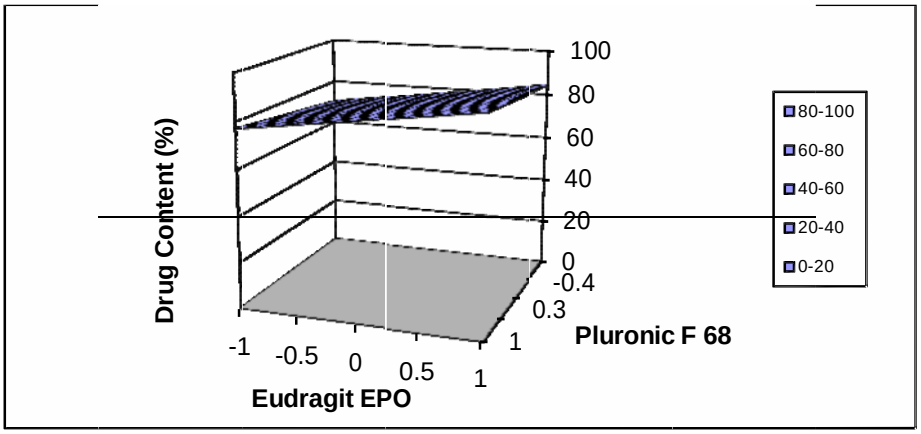


Fig. 9.9: Response surface plots showing effect of factorial variables on drug content

The results of drug content are shown in the Table 9.11. The drug content of the nanoparticle were in the range from 68.65 ±1.32 to 90.35 ±2.09% depends on the concentration of polymer added. The maximum drug content was found in formulation F6 with 90.35 ±2.09%. Drug content was increases with increasing in concentration of polymer added to the formulation.

9.2.3. Estimation of Drug Entrapment Efficiency:

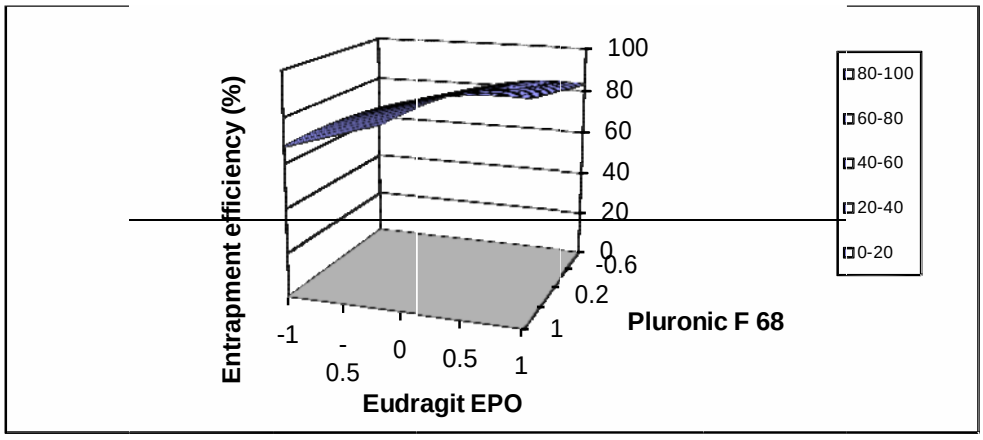


Fig. 9.10: Response surface plots showing effect of factorial variables on drug entrapment efficiency

Table 9.11: Comparison of drug content with entrapment efficiency of formulation F1-F9

S. No	Batch code	*Drug content (%)	*Drug entrapment efficiency (%)
1	F1	68.65±1.32	56.43±0.63
2	F2	72.92±0.44	62.39±1.15
3	F3	75.20±1.40	66.13±0.36
4	F4	79.35±1.43	74.50±0.97
5	F5	81.93±0.36	83.97±1.78
6	F6	90.35±2.09	93.33±1.92
7	F7	84.79±0.46	85.72±0.35
8	F8	86.55±1.95	89.58±0.63
9	F9	87.95±2.89	91.87±2.02

* All the values expressed as mean ± mean S.D., n=3

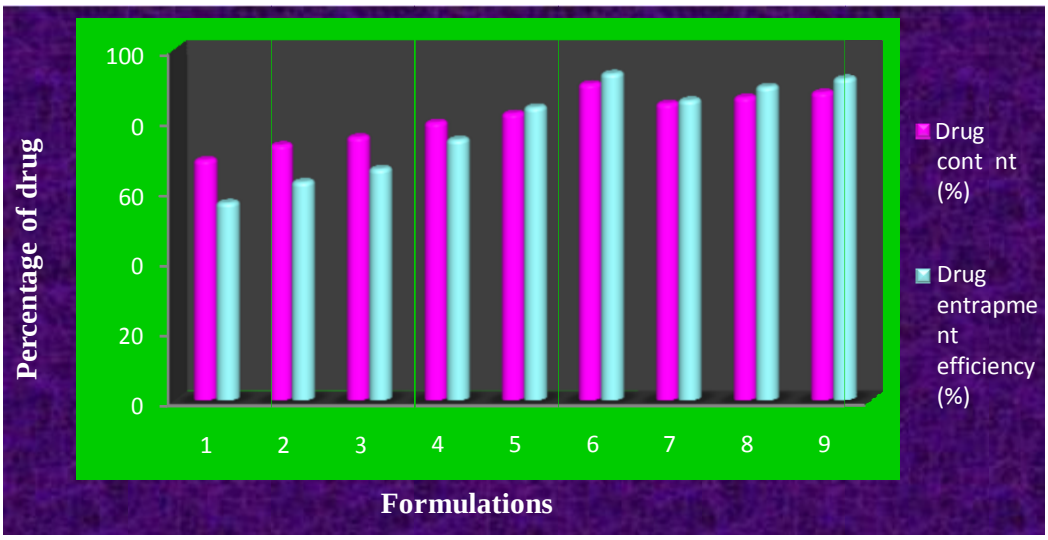


Fig. 9.11: Comparative graph of drug content and entrapment efficiency of formulation F1-F9

The entrapment efficiency of the acyclovir loaded pH sensitive nanoparticle was exhibit maximum in formulation F6 with 93.33±1.92%. Drug entrapment efficiency of the nanoparticle was in the range from 56.43±0.63 to 93.33±1.92%. The results were also in the Table 9.11. As the Eudragit®-EPO concentration increased from 100-200 mg the encapsulation efficiency was increased. When the Eudragit®-EPO concentration was maintained constant of 100 mg, the Pluronic® F-68 concentration was varied as 25 mg, 50 mg, and 100 mg the encapsulation efficiency was slightly increased. The results indicate the Eudragit®-EPO concentration plays a major role in drug entrapment efficiency rather than the Pluronic® F-68 concentration and shown in Figure 9.10.

It can be explained on the basis of lipophilic - lipophilic interaction between acyclovir and Eudragit®-EPO. Consequently with increase in the Eudragit®-EPO amount, Acyclovir gets preferentially dispersed in the internal organic phase.

Pluronic® F-68, also displayed similar trend and increase in encapsulation efficiency which can be due to the formulation of interpenetrated network chain between the hydrophobic portion of Pluronic® F-68 with Eudragit®-EPO during precipitation. The comparative results of drug content and entrapment were shown in Figure 9.11.

9.2.4 Development of Polynomial Equation:

From the data of Experimental design and Parameters Table 9.11 for factorial formulations F1 to F9, polynomial equations for two dependent variables (drug content and % drug entrapment efficiency) have been derived using PCP Disso 2000V3 software. The equation derived for drug content is:

$$Y1= 80.8544 + 7.0867X1 + 3.4517 X2 \quad \ldots 2$$

The equation derived for % drug entrapment efficiency is:

$$Y2=83.9333 + 13.7033 X1 + 5.7800X2 – 8.5800X1\ 2 \quad \ldots 3$$

In equation (2) positive sign for coefficient of X_2 indicates when concentration of stabilizer (Pluronic F-68) is increases and Positive sign for coefficient of X_1 indicate positive effect of polymer concentration (Eugragit®-EPO) on drug content.

In equation (3) positive sign for coefficient of X_1 indicates that the % drug entrapment increases when concentration of polymer (Eugragit®-EPO) increases and positive sign for coefficient of X_2 indicates that % drug entrapment of nanoparticles increases when concentration of stabilizer (Pluronic F-68) increases.

9.2.4.1. Response Surface Plots:

Graphical presentation of the data can help to show the relationship between response and independent variables. Graphs gave information similar to that of the mathematical equations obtained from statistical analysis. The response surface

graphs of drug content and % drug entrapment are presented in Fig.9.9, and 9.10 respectively. The response surface plots illustrated that as concentration of as polymer (Eugragit®-EPO) increases, the value of dependent variable i.e. drug content increases and as concentration of stabilizer (Pluronic F-68) increases the value of dependent variable i.e. drug content decreases. Similarly the response surface plots for % drug entrapment shows positive effects of independent variable i.e. polymer concentration (Eugragit®-EPO) and negative effect of other independent variable i. e. concentration of stabilizer (Pluronic F-68).

9.2.5 Fourier Transformer Infrared Spectroscopy (FTIR):

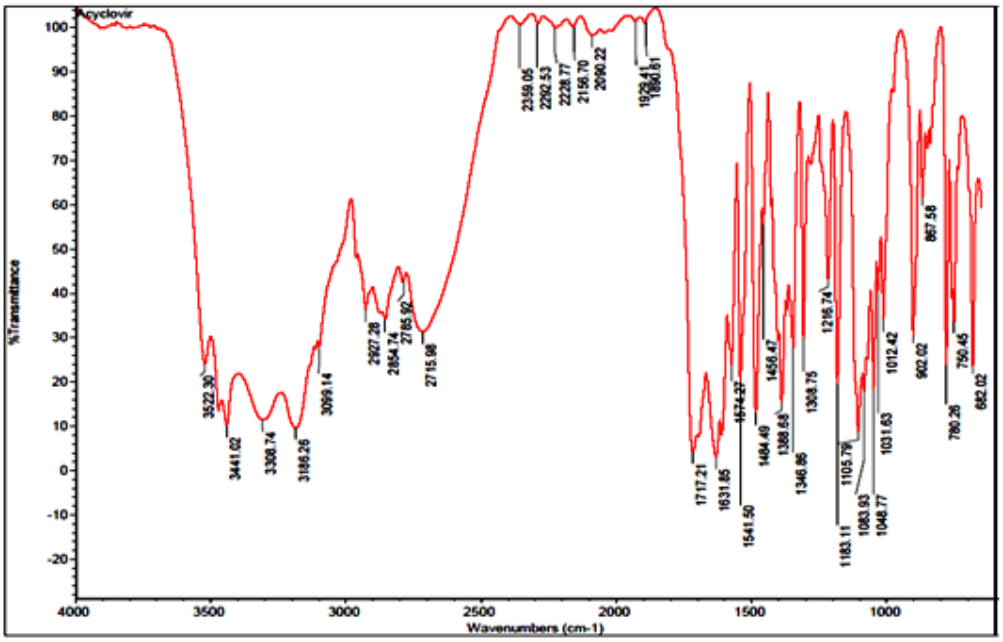


Fig. 9.12: FTIR Spectrum of pure Acyclovir

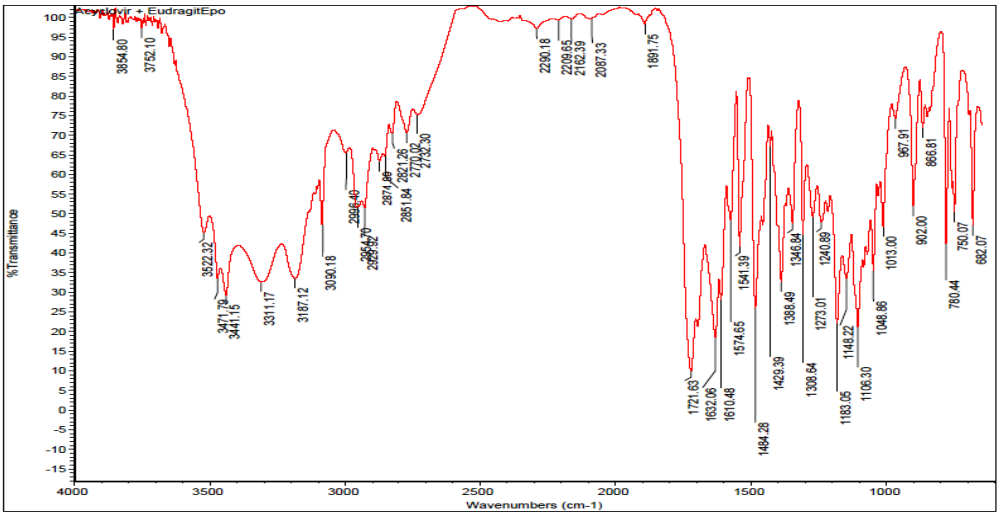


Fig. 9.13: FTIR Spectrum of Acyclovir and Eudragit®-EPO

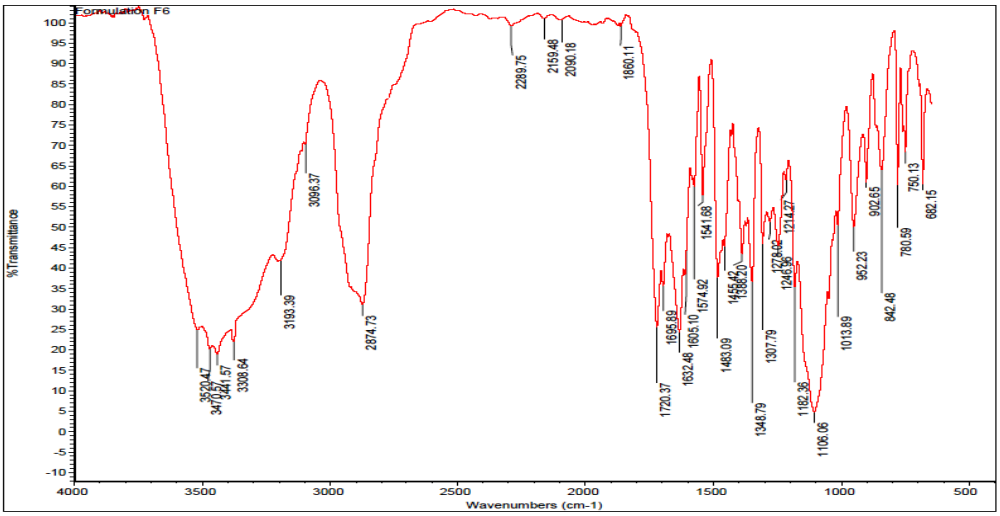


Fig. 9.14: FTIR Spectrum of lyophilized nanoparticle formulation F6

The possible drug and polymer interaction can be studied by FTIR Spectroscopy. According to Table 9.12 and Figure 9.12 to Figure 9.14 the major peaks observed in drug spectrum were also observed in spectrums of drug with

polymer, therefore it could indicate that there was no incompatibility between drug and polymer.

Table 9.12: Interpretation of FTIR spectrum

S. No.	Wave number (cm^{-1})	Functional group	Peak observed (Yes/No)			
			Range (cm^{-1})	Drug	Drug + Eudragit®-EPO	Formulation F6
1	3441.02	N-H stretching	3500-3200	Yes	Yes	Yes
2	3308.74	O-H stretching	3400-3200	Yes	Yes	Yes
3	3099.14	C-H stretching	3100-3000	Yes	Yes	Yes
4	1717.21	C=O stretching	1740-1690	Yes	Yes	Yes
5	1541.50	C=C stretching	1600-1430	Yes	Yes	Yes
6	1308.75	CH2-Wagging and twisting	1350-1150	Yes	Yes	Yes
7	1216.74	Aryl alkyl ether	1275-1200	Yes	Yes	Yes
8	1105.79	C-O stretching	1200-1100	Yes	Yes	Yes
9	1083.93	C-O-C stretching	1150-1070	Yes	Yes	Yes

9.2.6. Differential Scanning Calorimetory (DSC) Analysis:

Table 9.13: Data for DSC thermogram parameters

S.No.	DSC thermogram sample	Onset temperature (°C)	Peak temperature (°C)	Endset Temperature (°C)
1	Acyclovir	251.13	254.75	257.83
2	Acyclovir + Eudragit®-EPO	122.52	124.89	135.54
3	Formuation F6	248.33	257.29	258.75

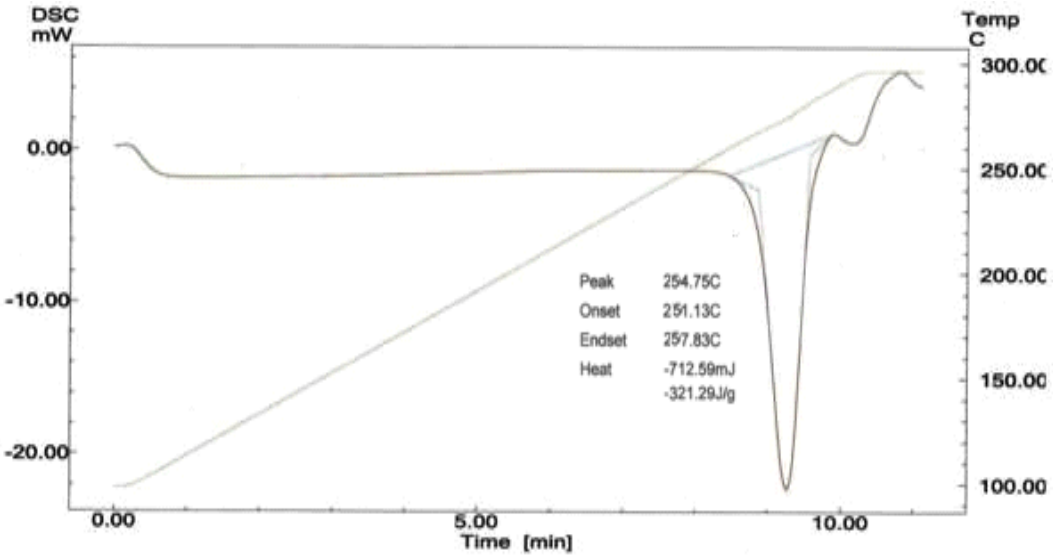


Fig. 9.15: DSC Curve of pure Acyclovir

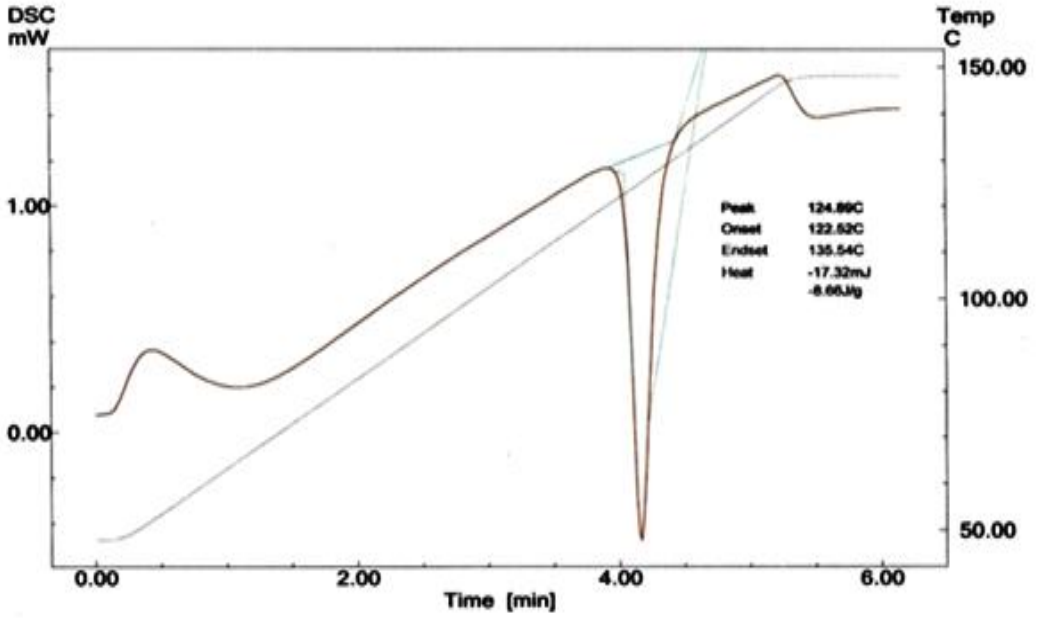


Fig. 9.16: DSC Curve of acyclovir and Eudragit®-EPO

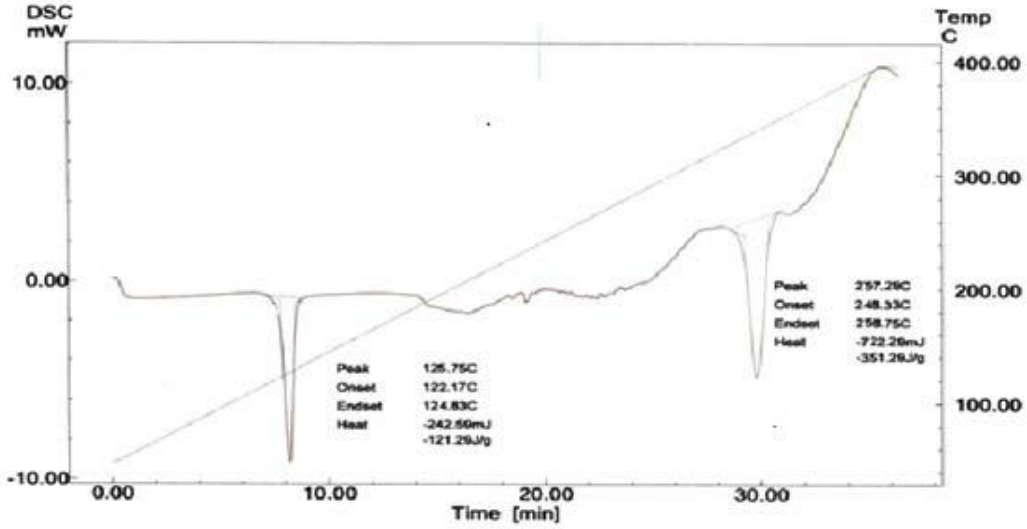


Fig. 9.17: DSC Curve of lyophilized nanoparticle formulation F6

Figure 9.15, 9.16, and 9.17 shows the DSC thermograms of Acyclovir, Eudragit®-EPO and lyophilized nanoparticle suspension respectively. Acyclovir

exhibits a sharp melting endothermic peak at 254.75ºC (ΔH 321.29 J/g), where as Eudragit®-EPO showed a broad melting endrotherm peak at 124.89ºC (ΔH 8.66 J/g). The thermogram of lyophilized nanoparticle suspension displayed sharp endotherm at 267.29^0C (ΔH 351.20 J/g), corresponding to Eudragit®-EPO but no drug peak it explains monotetic behavior of the system, where drug gets completely dissolve in below it temperature. It is evident that there was no chemical interaction between drug and polymer. The results are shown in table 9.13 and Figure 9.15, 9.16, and 9.17

9.2.7. Scanning electron microscopy (SEM) analysis:

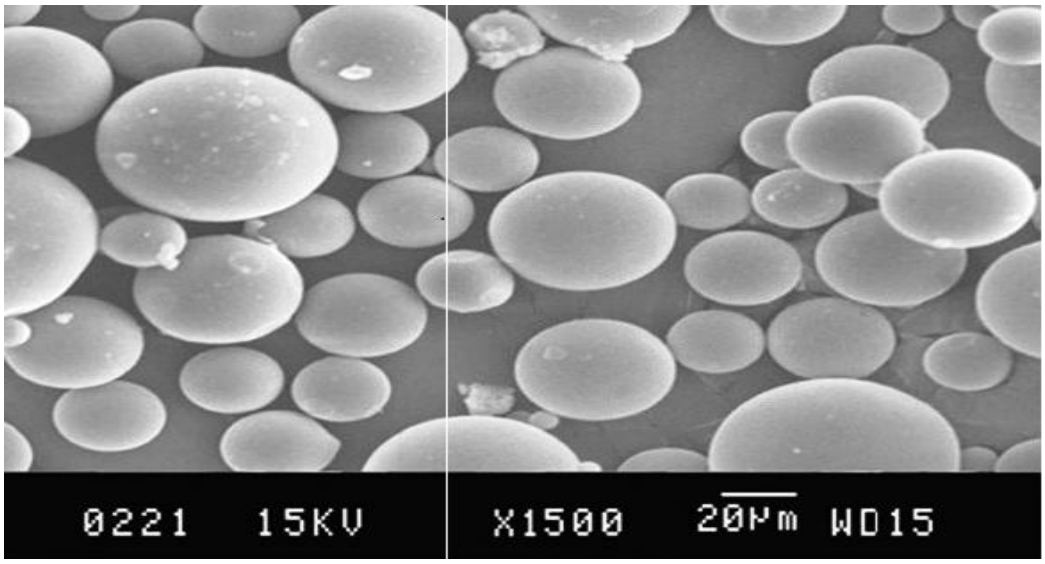


Fig. 9.18: SEM Photograph of lyophilized nanoparticle formulation F6

SEM photograph of optimized formulation F6 were shown in Figure 9.18. Acyclovir nanoparticle has shown smooth and spherical shape. The results is depends on the ratio of surfactant and polymer used in the optimized formulation F6.

9.2.8. Zeta Potential Measurement:

Temperature (°C):	25.0	**Zeta Runs:**	30
Count Rate (kcps):	75.1	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta cell	**Attenuator:**	7

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	30.7	**Peak 1:**	30.7	100.0	3.89
Zeta Deviation (mV):	3.89	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.474	**Peak 3:**	0.00	0.0	0.00

Result quality : Good

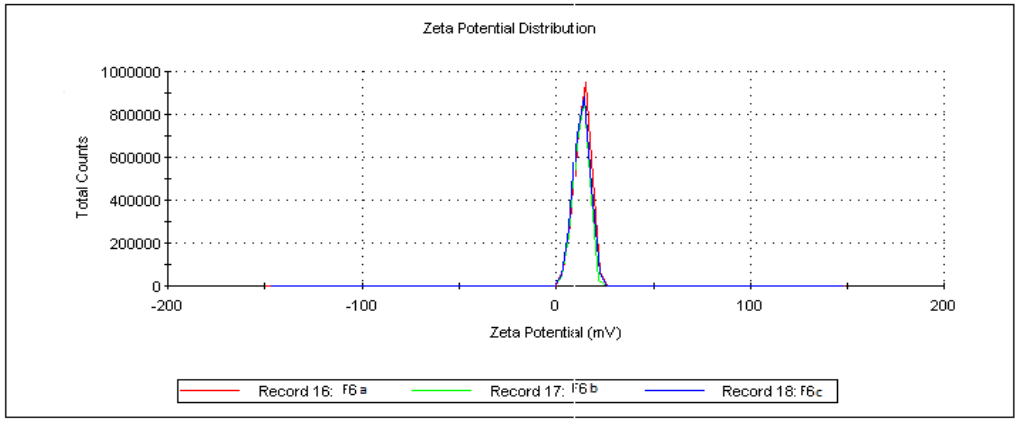


Fig. 9.19: Plot of Zeta Potential distribution for the formulation F6

The zeta potential values of the nanoparticle suspension had 30.7 ±2.4 mV. The formulation F6 exhibited strongly positive zeta potential values due to polycationic Eudragit® EPO comprising of various ammonium groups. The increasing zeta potential values in initial batches may be attributed to Eudragit® EPO available at the surface of the particles due to high viscosity of the external aqueous phase.

9.3. *In-Vitro* Drug Release Study:

The release rate of nanoparticle depend upon

i) desorption of the surface-bound/adsorbed drug; ii) diffusion through the nanopaticle matrix; iii) diffusion (in case of nanocapsule) through the polymer wall;

iv) nanoparticle matrix erosion: and v) a combined erosion/diffusion process. Thus, diffusion and biodegradation govern the process of drug release.

The mechanism of drug release from nanoparticle is determined by different physical–chemical phenomena. The exponent n has been proposed as indicative of the release mechanism. In this context, $n \leq 0.43$ indicates Fickian release and $n = 1$ indicates a purely relaxation controlled delivery. Intermediate values $0.43 < n < 1$ indicate an anomalous behavior (non-Fickian kinetics corresponding to coupled diffusion/polymer relaxation.

The average percentage release was fitted into different release models: zero order, first order, Higuchi's square root plot and Korsmeyer-Peppas models. The models giving a correlation coefficient close to unity were taken as the order of release In vitro drug release data of all selected factorial formulations was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equations, Higuchi's, and Korsmeyer-Peppas models to ascertain the mechanism of drug release. It is evident that values of 'r' of factorial formulation F6 was found to be 0.997. This data reveals that drug release follows zero order release kinetics with non-fickian diffusion mechanism Drug release for selected factorial formulation F6 are 94.46±0.57% at the end of 13 hours. Kinetic exponent 'n' for these formulations indicate diffusion through the nanoparticle matrix as well matrix erosion. Finally, it can be concluded that the different drug release rates may be attributed to different sizes of the nanoparticle. It is expected as the particle size of Eudragit®-EPO nanoparticle is smaller, their surface areas will be more and the drug release is faster

Table 9.14: *In-vitro* drug release data of formulation F1

Time (hours)	DR* (%)	Amount (mg)	% DE	MDT
0	0.00	0.00	0.00	0.00
1	13.37±1.06	1.34	6.00	0.50
2	18.42±1.73	1.84	11.20	0.92
3	23.80±1.64	2.38	15.21	1.22
4	31.52±1.22	3.15	18.43	1.58
5	36.95±1.79	3.70	21.37	2.02
6	41.41±1.51	4.14	24.20	2.44
7	50.05±1.06	5.01	27.21	3.17
8	57.50±2.24	5.75	30.39	3.62
9	65.87±1.20	6.59	33.79	4.43
10	70.81±1.48	7.08	37.27	4.73
11	77.39±0.81	7.74	40.59	5.18
12	81.47±2.67	8.15	43.66	5.30

* All the values expressed as mean ± mean S.D., n=3

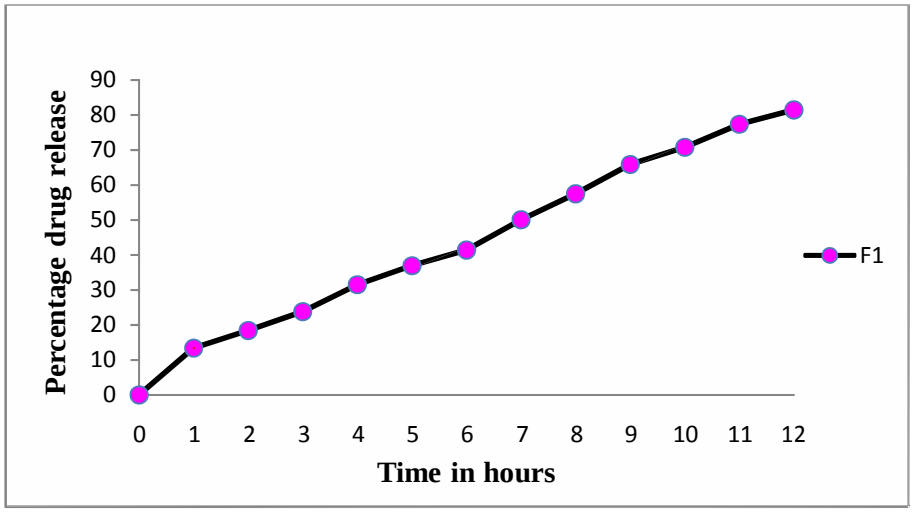


Fig. 9.20: *In vitro* drug release profile of formulation F1

Table 9.15: *In-vitro* drug release data of formulation F2

Time (hours)	DR* (%)	Amount (mg)	% DE	MDT
0	0.00	0.00	0.00	0.00
1	10.32±1.31	1.03	5.54	0.50
2	18.85±0.86	1.89	9.91	0.87
3	25.49±1.33	2.55	13.61	1.34
4	29.67±0.52	2.97	16.96	1.70
5	37.66±0.68	3.77	20.22	2.28
6	42.77±1.39	4.28	23.68	2.83
7	51.25±1.85	5.13	27.24	3.35
8	55.16±2.17	5.52	30.71	3.75
9	66.57±0.68	6.66	34.21	4.37
10	69.40±0.65	6.94	37.53	4.51
11	79.07±1.80	7.91	40.87	5.40
12	84.07±1.30	8.41	44.32	5.68

* All the values expressed as mean ± mean S.D., n=3

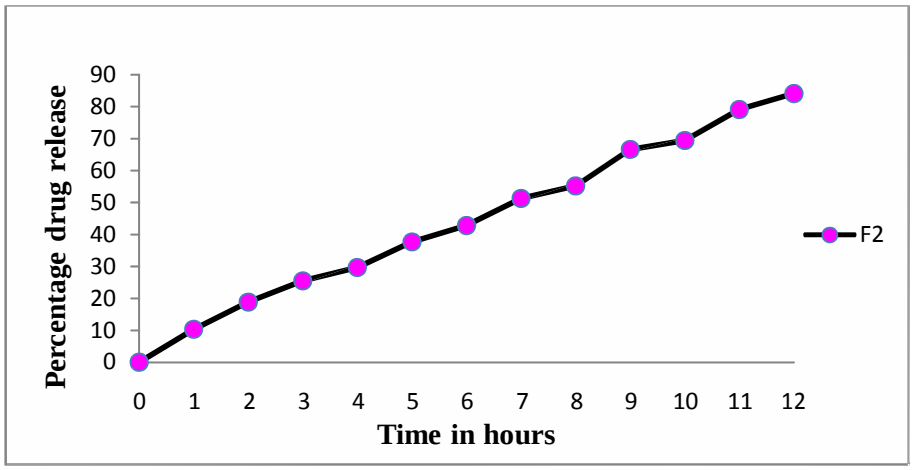


Fig. 9.21: *In vitro* drug release profile of formulation F2

Table 9.16: *In-vitro* drug release data of formulation F3

Time (hours)	DR* (%)	Amount (mg)	% DE	MDT
0	0.00	0.00	0.00	0.00
1	13.64±0.98	1.36	5.60	0.50
2	19.56±1.06	1.96	10.38	0.91
3	23.42±2.20	2.34	14.42	1.33
4	30.70±2.81	3.07	18.24	1.82
5	37.77±1.39	3.78	21.62	2.06
6	42.17±2.78	4.22	24.37	2.30
7	49.45±1.99	4.95	27.10	3.00
8	55.81±1.05	5.58	30.17	3.68
9	64.29±2.13	6.43	33.59	4.43
10	70.54±2.62	7.05	37.09	4.77
11	72.82±2.62	7.28	40.22	4.86
12	84.62±1.47	8.46	43.34	5.76
13	87.28±0.98	8.73	46.56	6.03

* All the values expressed as mean ± mean S.D., n=3

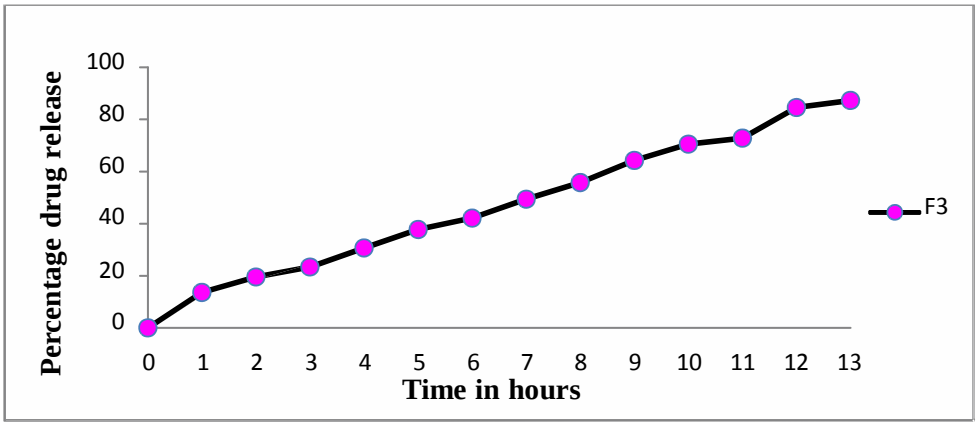


Fig. 8.22: *In vitro* drug release profile of formulation F3

Table 9.17: *In-vitro* drug release data of formulation F4

Time (hours)	DR* (%)	Amount (mg)	% DE	MDT
0	0.00	0.00	0.00	0.00
1	12.28±0.41	1.23	5.63	0.50
2	17.60±1.24	1.76	10.33	0.90
3	25.70±1.07	2.57	14.32	1.33
4	31.84±1.22	3.18	17.79	1.68
5	40.32±1.31	4.03	21.20	2.29
6	44.24±0.20	4.42	24.48	2.56
7	53.15±1.06	5.32	27.81	3.32
8	57.28±1.24	5.73	31.19	3.61
9	65.60±1.83	6.56	34.61	4.35
10	68.69±1.40	6.87	38.04	4.64
11	76.41±1.72	7.64	41.39	5.23

* All the values expressed as mean ± mean S.D., n=3

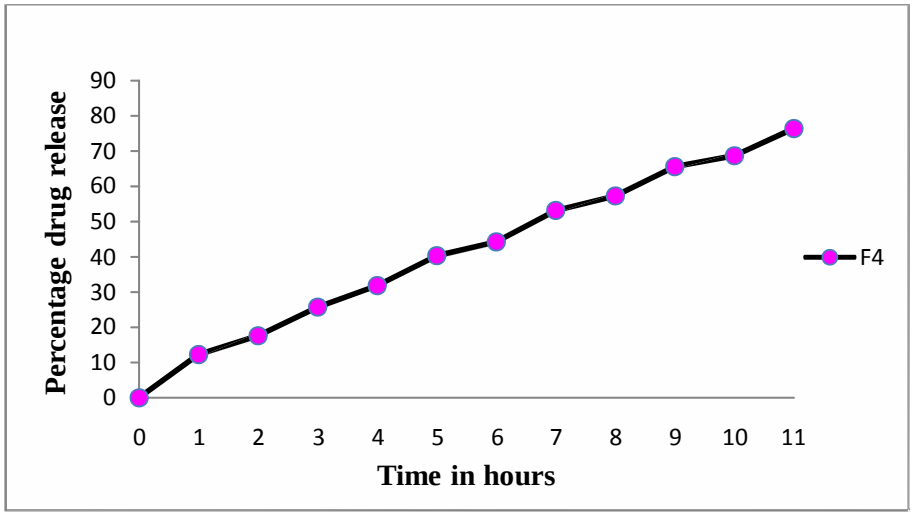


Fig. 9.23: *In vitro* drug release profile of formulation F4

Table 9.18: *In-vitro* drug release data of formulation F5

Time (hours)	DR* (%)	Amount (mg)	% DE	MDT
0	0.00	0.00	0.00	0.00
1	14.23±1.08	1.42	6.86	0.50
2	21.52±0.93	2.15	12.26	0.86
3	27.88±1.73	2.79	16.18	1.16
4	33.53±1.40	3.35	19.76	1.72
5	38.80±1.42	3.88	23.09	1.98
6	47.93±1.96	4.79	26.67	2.86
7	53.09±0.49	5.31	30.32	3.04
8	60.49±1.74	6.05	33.54	3.42
9	66.63±0.71	6.66	36.80	4.07
10	73.75±0.73	7.38	40.12	4.49
11	79.13±1.66	7.91	43.39	4.99
12	85.54±1.14	8.55	46.59	5.36

* All the values expressed as mean ± mean S.D., n=3

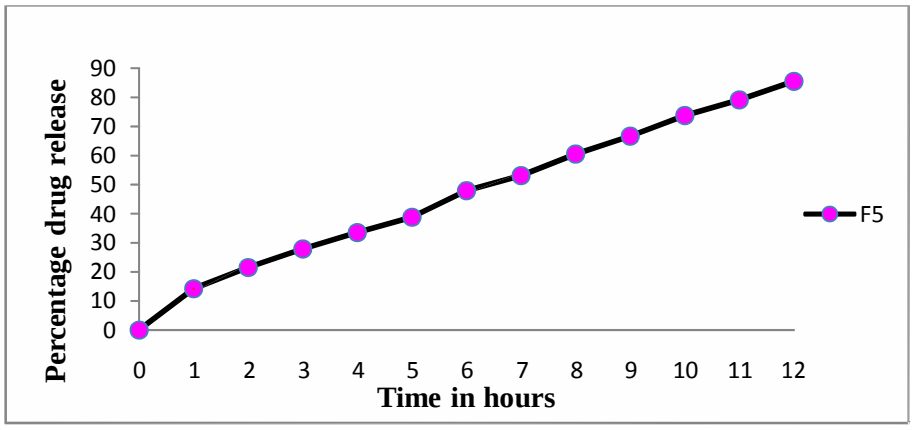


Fig. 9.24: *In vitro* drug release profile of formulation F5

Table 9.19: *In-vitro* drug release data of formulation F6

Time (hours)	DR* (%)	Amount (mg)	% DE	MDT
0	0.00	0.00	0.00	0.00
1	9.40±0.65	0.94	4.80	0.50
2	15.87±1.06	1.59	8.38	0.83
3	22.82±0.73	2.28	11.72	1.44
4	31.63±0.57	3.16	15.70	2.09
5	38.86±0.52	3.89	19.76	2.47
6	44.45±1.14	4.45	23.36	2.79
7	51.30±1.55	5.13	26.80	3.34
8	59.56±0.93	5.96	30.35	3.89
9	66.79±1.42	6.68	33.99	4.44
10	74.13±0.81	7.41	37.69	4.97
11	81.52±2.17	8.15	41.45	5.51
12	87.82±1.14	8.78	45.11	5.82
13	94.46±0.57	9.45	48.68	6.36

* All the values expressed as mean ± mean S.D., n=3

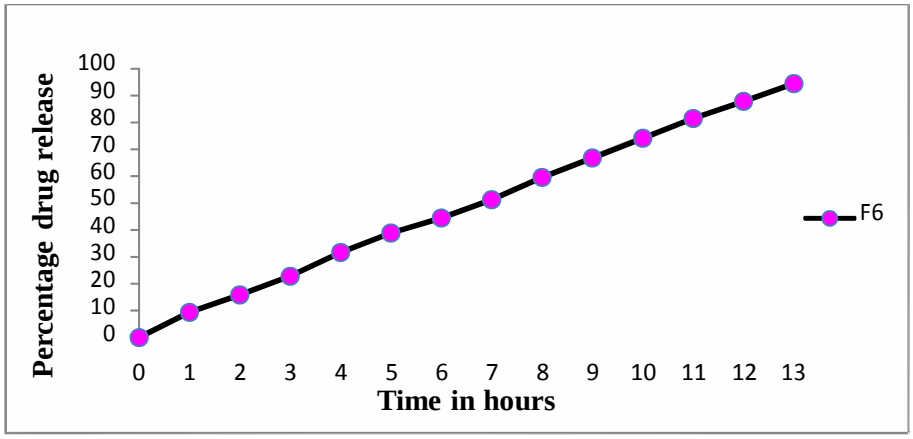


Fig. 9.25: *In vitro* drug release profile of formulation F6

Table 9.20: *In-vitro* drug release data of formulation F7

Time (hours)	DR* (%)	Amount (mg)	% DE	MDT
0	0.00	0.00	0.00	0.00
1	12.77±0.98	1.28	6.12	0.50
2	19.67±0.65	1.97	11.36	0.91
3	25.65±0.65	2.57	15.29	1.19
4	33.69±0.98	3.37	18.76	1.72
5	37.06±1.94	3.71	21.89	1.95
6	44.72±1.14	4.47	25.04	2.72
7	53.64±1.47	5.36	28.73	3.41
8	57.61±1.27	5.76	32.16	3.44
9	64.51±0.98	6.45	35.36	4.14
10	72.50±1.47	7.25	38.76	4.72

* All the values expressed as mean ± mean S.D., n=3

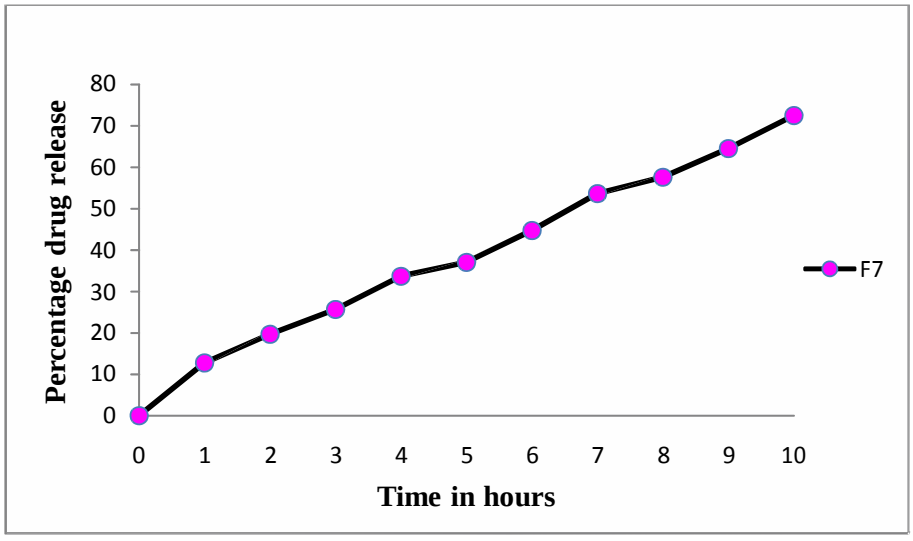


Fig. 9.26: *In vitro* drug release profile of formulation F7

Table 9.21: *In-vitro* drug release data of formulation F8

Time (hours)	DR* (%)	Amount (mg)	% DE	MDT
0	0.00	0.00	0.00	0.00
1	15.38±1.39	1.54	8.21	0.50
2	20.59±1.00	2.06	13.44	0.72
3	28.42±1.22	2.84	17.38	1.24
4	32.22±1.14	3.22	20.68	1.38
5	40.38±1.07	4.04	23.68	2.03
6	49.83±1.55	4.98	27.31	2.80
7	57.88±0.73	5.79	31.20	3.23
8	62.71±1.08	6.27	34.85	3.57
9	71.25±1.14	7.13	38.51	4.22
10	78.04±0.59	7.80	42.17	4.57

* All the values expressed as mean ± mean S.D., n=3

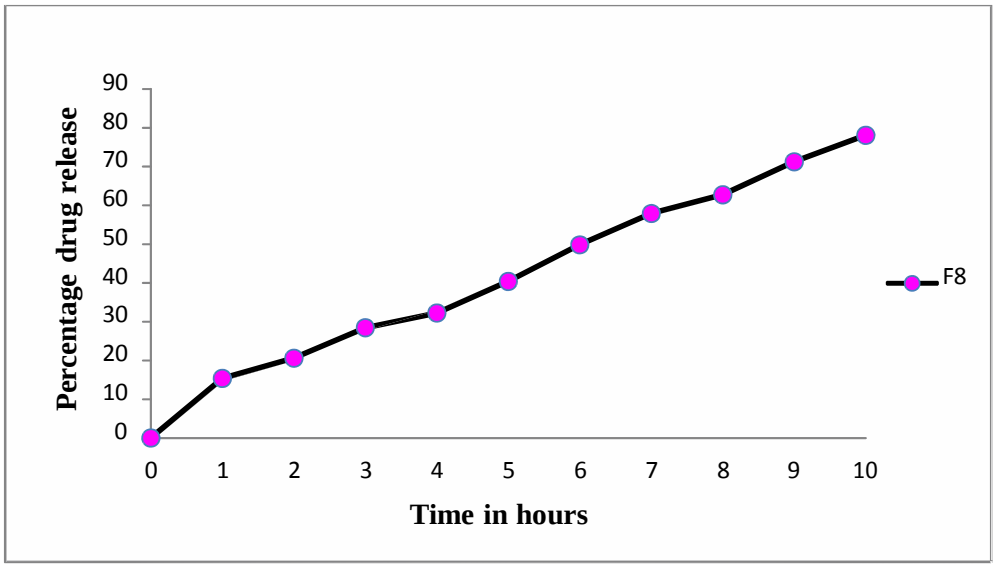


Fig. 9.27: *In vitro* drug release profile of formulation F8

Table 9.22: *In-vitro* drug release data of formulation F9

Time (hours)	DR* (%)	Amount (mg)	% DE	MDT
0	0.00	0.00	0.00	0.00
1	12.66±0.98	1.27	6.03	0.50
2	21.73±2.04	2.17	11.42	0.94
3	27.06±0.90	2.71	15.60	1.23
4	31.46±1.14	3.15	19.11	1.67
5	37.82±1.73	3.78	22.14	1.90
6	45.70±0.50	4.57	25.31	2.75
7	51.63±0.50	5.16	28.74	3.11
8	58.86±0.57	5.89	32.03	3.61
9	64.29±2.13	6.43	35.19	3.94
10	67.39±1.51	6.74	38.06	4.15
11	74.83±1.14	7.48	40.94	4.95
12	82.34±1.42	8.23	44.09	5.63

* All the values expressed as mean ± mean S.D., n=3

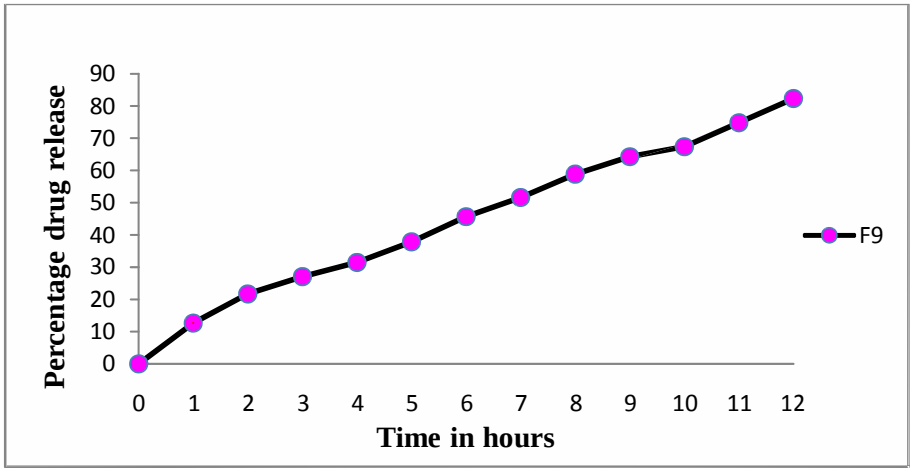


Fig. 9.28: *In vitro* drug release profile of formulation F9

Table 9.23: Comparative drug release data for all formulations

Time in hours	Formulation code								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	13.37±1.06	10.32±1.39	13.64±0.98	12.28±0.41	14.23±1.08	9.40±0.65	12.77±0.98	15.38±1.39	12.66±0.98
2	18.42±1.73	18.85±0.86	19.56±1.06	17.60±1.24	21.52±0.93	15.87±1.06	19.67±0.65	20.59±1.00	21.73±2.04
3	23.80±1.64	25.49±1.33	23.42±2.20	25.70±1.07	27.88±1.73	22.82±0.73	25.65±0.65	28.42±1.22	27.06±0.90
4	31.52±1.22	29.67±0.52	30.70±2.81	31.84±1.22	33.53±1.40	31.63±0.57	33.69±0.98	32.22±1.14	31.46±1.14
5	36.95±1.79	37.66±0.68	37.77±1.39	40.32±1.31	38.80±1.42	38.86±0.52	37.06±1.94	40.38±1.07	37.82±1.73
6	41.41±1.51	42.77±1.39	42.17±2.78	44.24±2.20	47.93±1.96	44.45±1.14	44.72±1.14	49.83±`1.55	45.70±0.50
7	50.05±1.06	51.25±1.85	49.45±1.99	53.15±1.06	53.09±0.49	51.30±1.55	53.64±1.47	57.88±0.73	51.63±0.50
8	57.50±2.24	55.16±2.17	55.81±1.05	57.28±1.24	60.49±1.74	59.56±0.93	57.61±1.27	62.71±1.08	58.86±0.57
9	65.87±1.20	66.57±0.68	64.29±2.13	65.60±1.83	66.63±1.71	66.79±1.42	64.51±0.98	71.25±1.14	64.29±2.13
10	70.81±1.48	69.40±0.65	70.54±2.62	68.69±1.40	73.75±0.73	74.13±0.81	72.50±1.47	78.04±0.59	67.39±1.51
11	77.39±0.81	79.07±1.80	72.82±2.62	76.41±1.72	79.13±0.66	81.52±2.17	-	-	74.83±1.14
12	81.47±2.67	84.07±1.30	84.62±1.47	-	85.54±1.14	87.82±1.14	-	-	82.34±1.42
13	-	-	87.28±0.98	-	-	94.46±0.57	-	-	-

All the values expressed as mean ± mean S.D., n=3

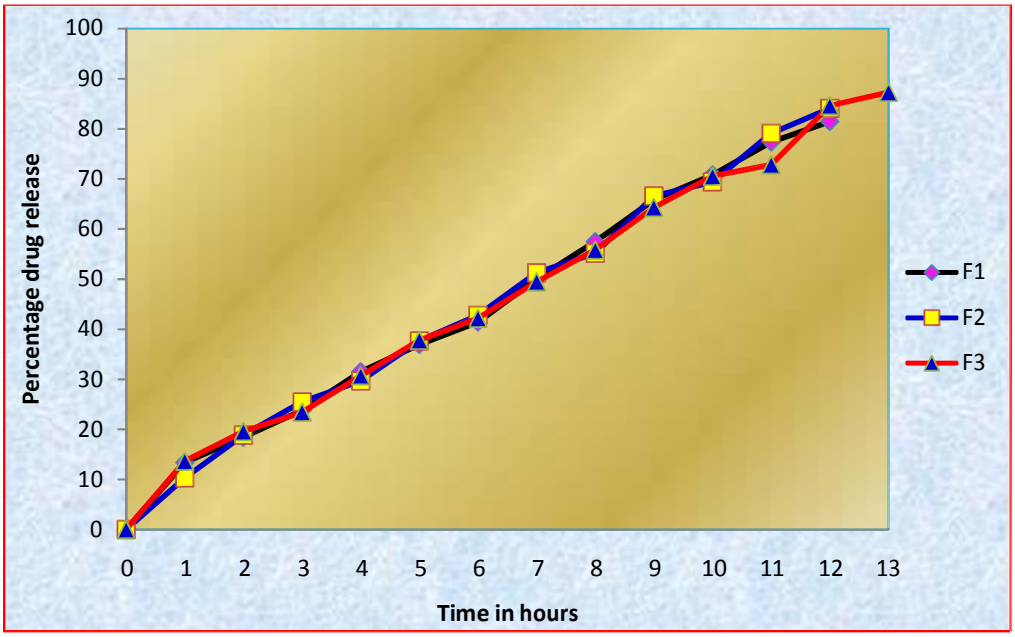


Fig. 9.29: Comparative drug release profile between formulations F1 to F3

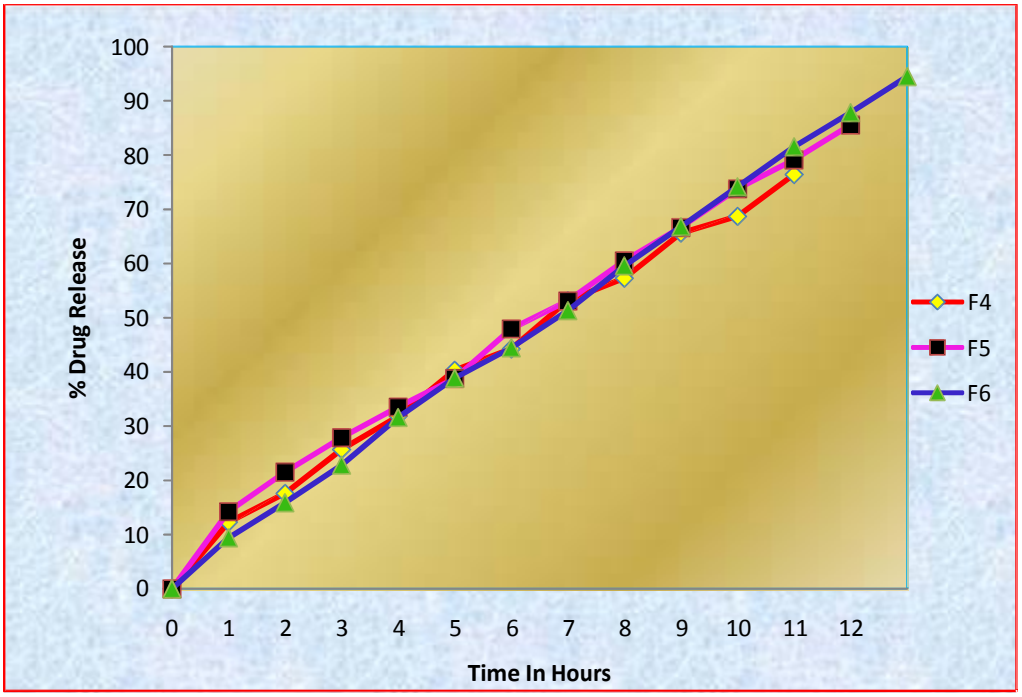


Fig. 9.30: Comparative drug release profile between formulations F4 to F6

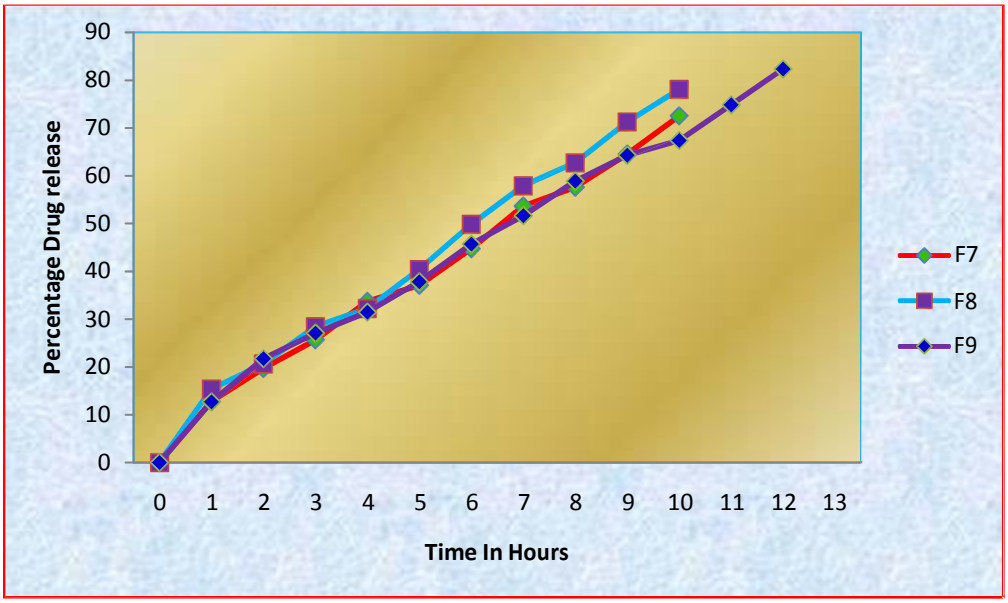


Fig. 9.31: Comparative drug release profile between formulations F7 to F9

9.3.1 Kinetics of *in-vitro* drug release:

Table 9.24: Kinetics of *in-vitro* drug release profile for all Formulation

Formulation code	Zero order R^2 value	First order R^2 value	Higuchi's R^2 value	Peppas		Best fit
				R^2 value	n value	
F1	0.994	0.943	0.937	0.981	0.952	Zero order
F2	0.995	0.920	0.935	0.995	0.929	Zero order
F3	0.994	0.905	0.938	0.980	0.884	Zero order
F4	0.993	0.970	0.981	0.992	0.960	Zero order
F5	0.989	0.937	0.952	0.990	0.937	Peppas
F6	0.997	0.835	0.931	0.995	0.963	Zero order
F7	0.992	0.968	0.947	0.991	0.918	Zero order
F8	0.992	0.953	0.940	0.976	0.856	Zero order
F9	0.991	0.950	0.955	0.993	0.907	Peppas

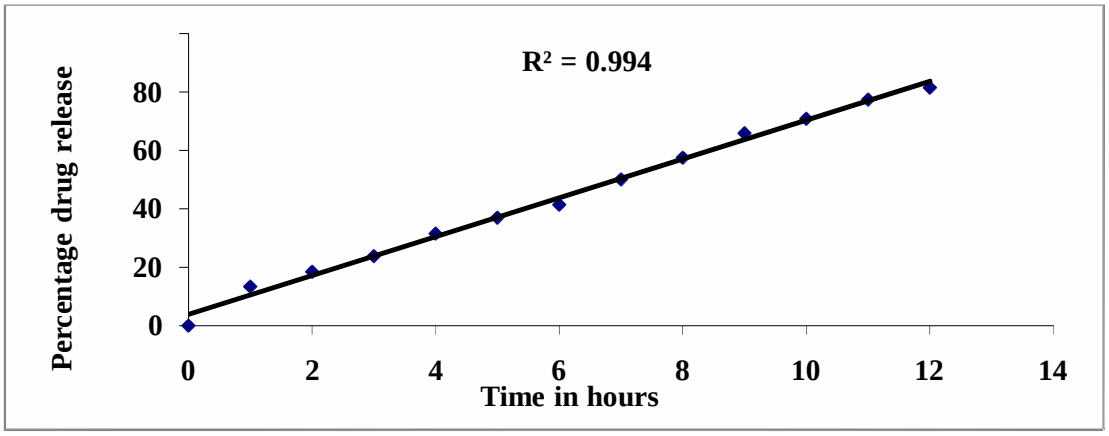


Fig. 9.32: Best fit model for formulation F1 (Zero order)

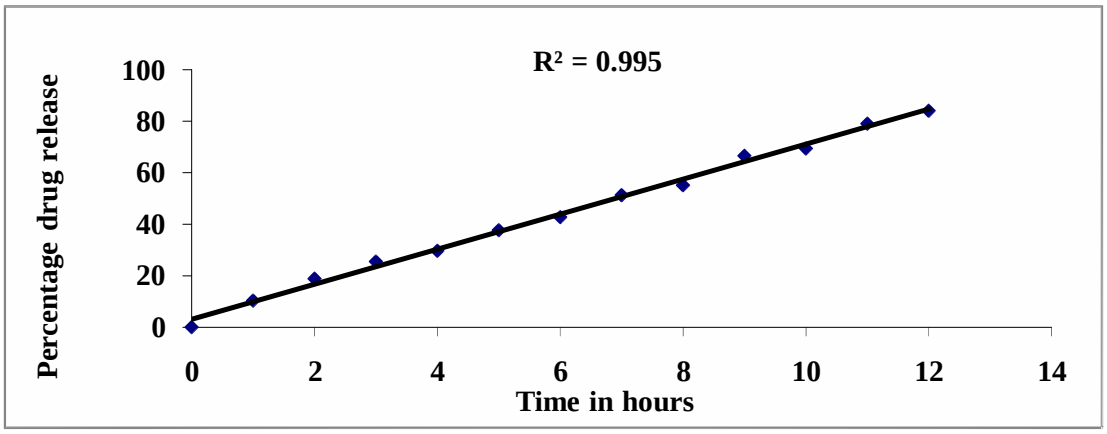


Fig. 9.33: Best fit model for formulation F2 (Zero order)

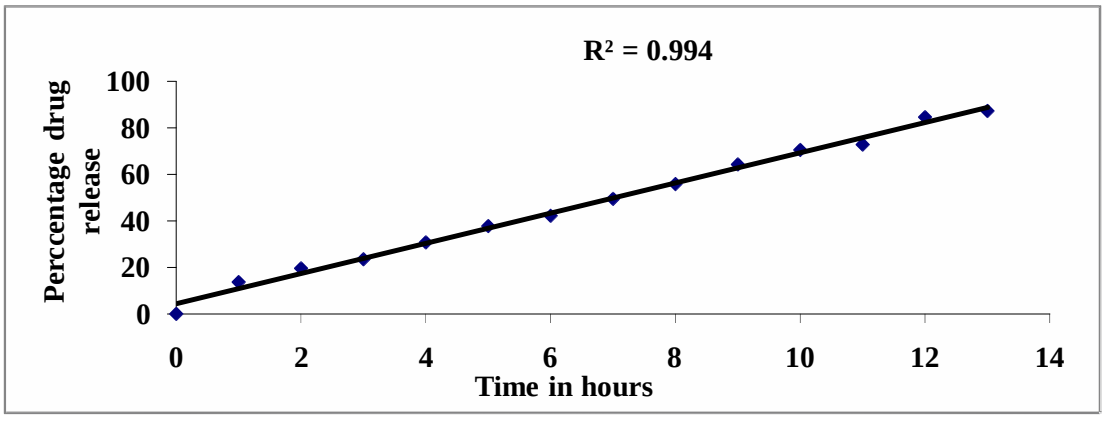


Fig. 9.34: Best fit model for formulation F3 (Zero order)

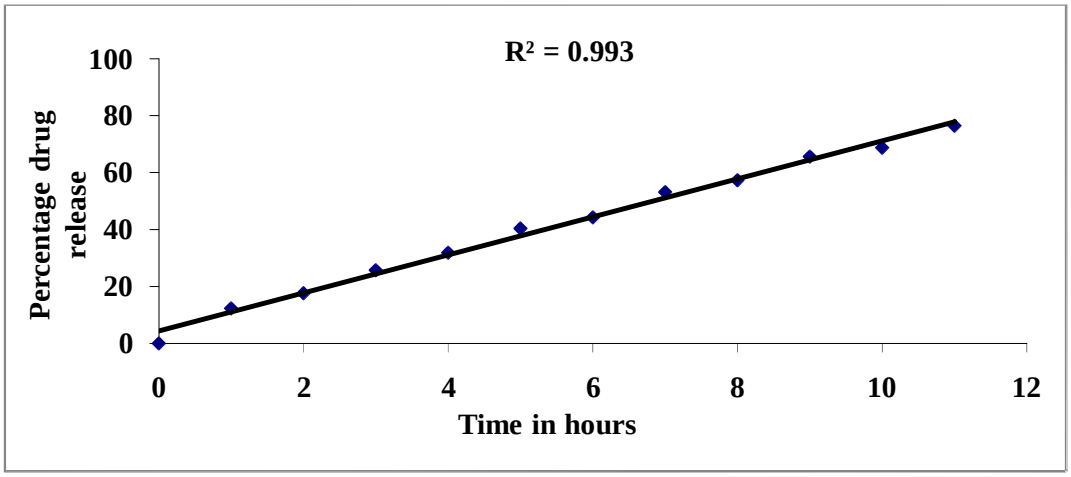


Fig. 9.35: Best fit model for formulation F4 (Zero order)

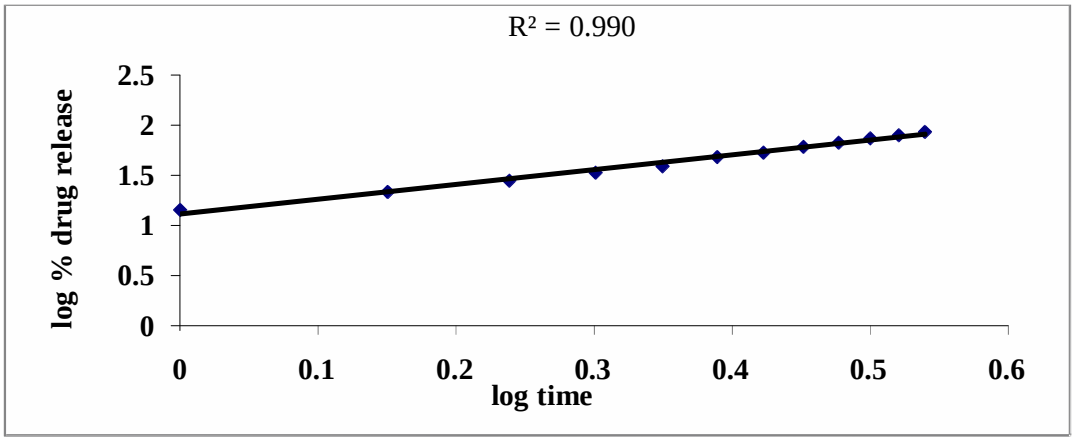


Fig. 9.36: Best fit model for formulation F5 (Peppas)

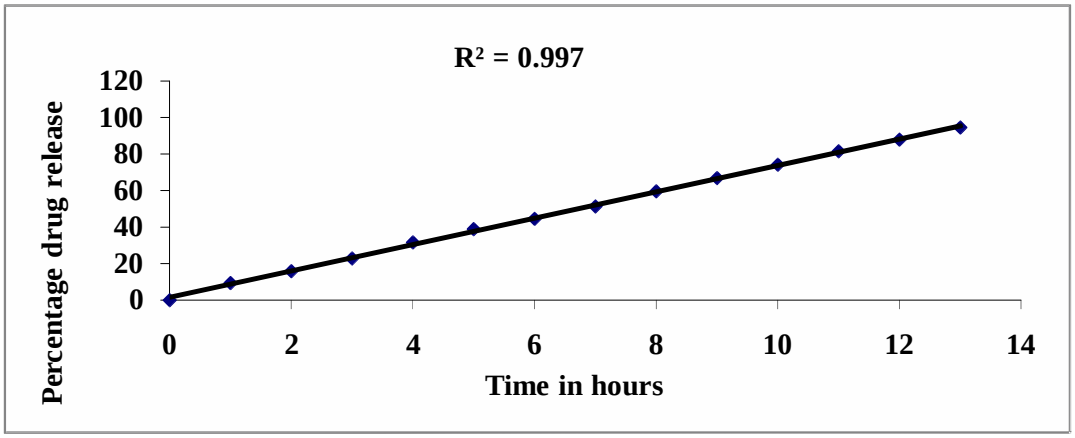


Fig. 9.37: Best fit model for formulation F6 (Zero order)

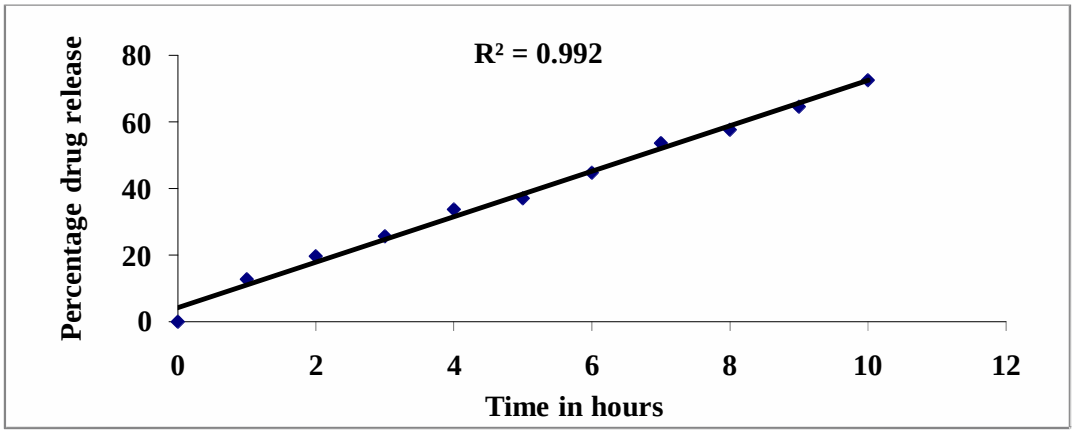


Fig. 9.38: Best fit model for formulation F7 (Zero order)

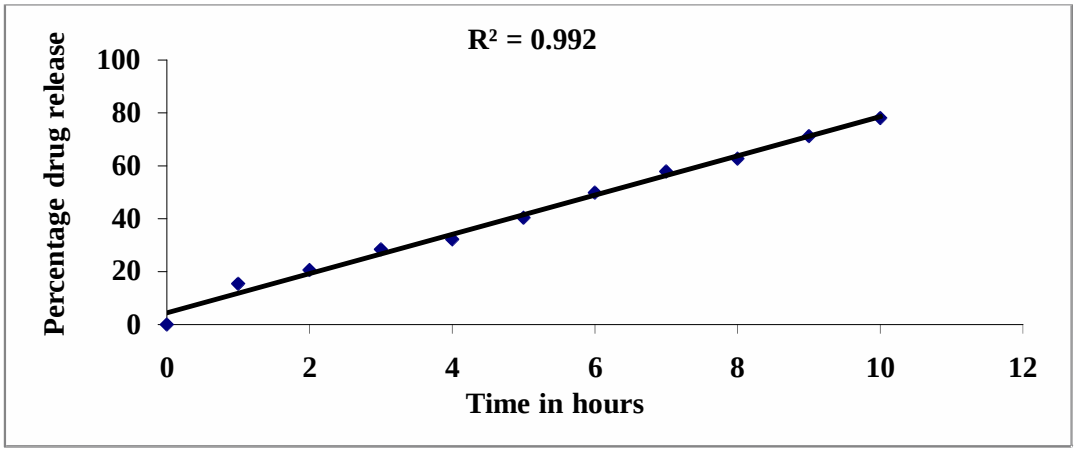


Fig. 9.39: Best fit model for formulation F8 (Zero order)

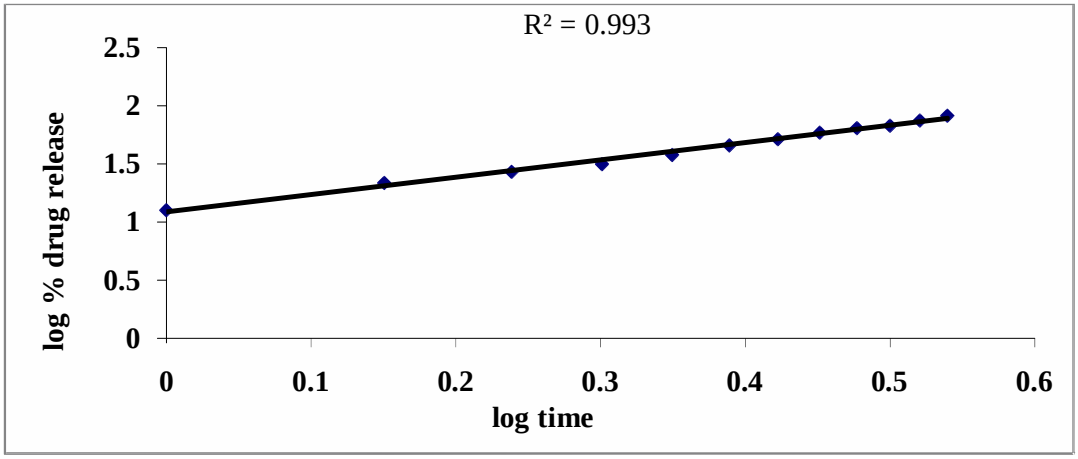


Fig. 9.40: Best fit model for formulation F9 (Peppas)

9.4. Stability study:

Table 9.25: Drug content and entrapment efficiency after 90 days storage of formulation F6

S.No.	Time	*Drug content		*Entrapment efficiency	
		4 ± 1^0C	**$25^0 \pm 2^0$C / 60 ± 5 % RH**	**4 ± 1^0C**	**$25^0 \pm 2^0$C / 60 ± 5 % RH**
1	0 Days	90.35±2.09	90.35±2.09	93.33±1.92	93.33±1.92
2	30 Days	90.23±0.70	90.00±1.47	93.03±0.49	92.86±0.26
3	60 Days	90.05±0.35	89.59±0.53	92.63±0.20	91.69±0.35
4	90 Days	89.65±0.70	88.24±0.66	92.16±0.10	90.70±0.44

* All the values expressed as mean ± mean S.D., n=3

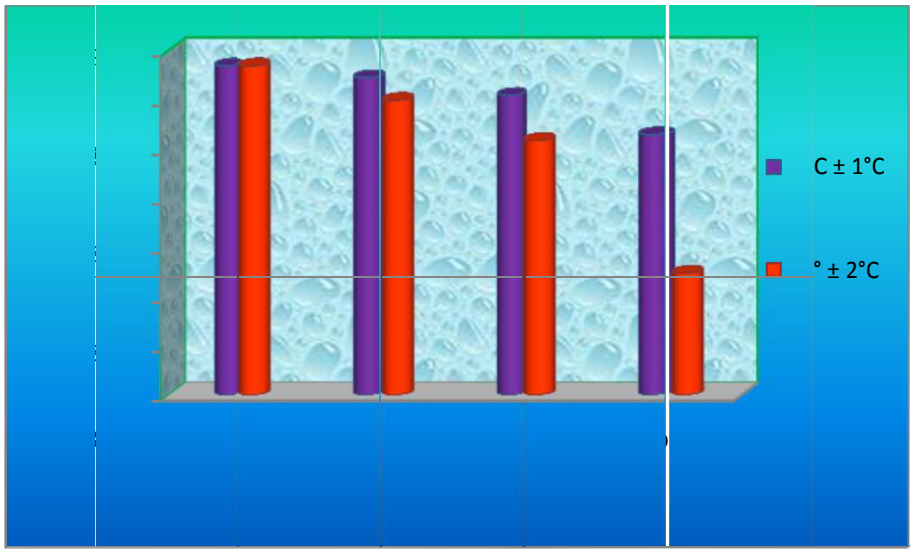


Fig. 9.41: Stability study (drug content) of lyophilized Nanoparticle formulation F6

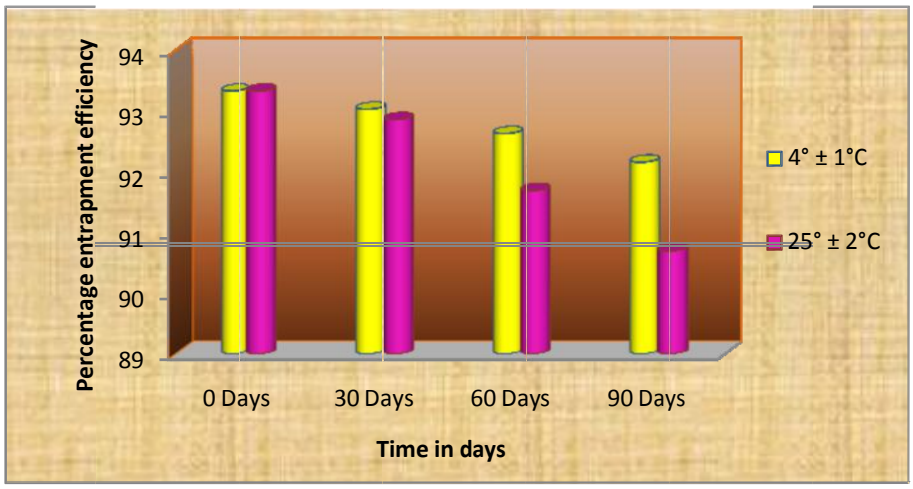


Fig. 9.42: Stability study (drug entrapment efficiency) of lyophilized nanoparticle formulation F6

The results is indicates that there was no change in appearance and settling behavior when observed visually. The drug content and entrapment efficiency after 90 days of the stability testing at different storage condition are shown in Table 9.25 and Figure 9.41 and 9.42. By comparing this data with initial data it was observed that there is overall decrease in % entrapment efficiency.

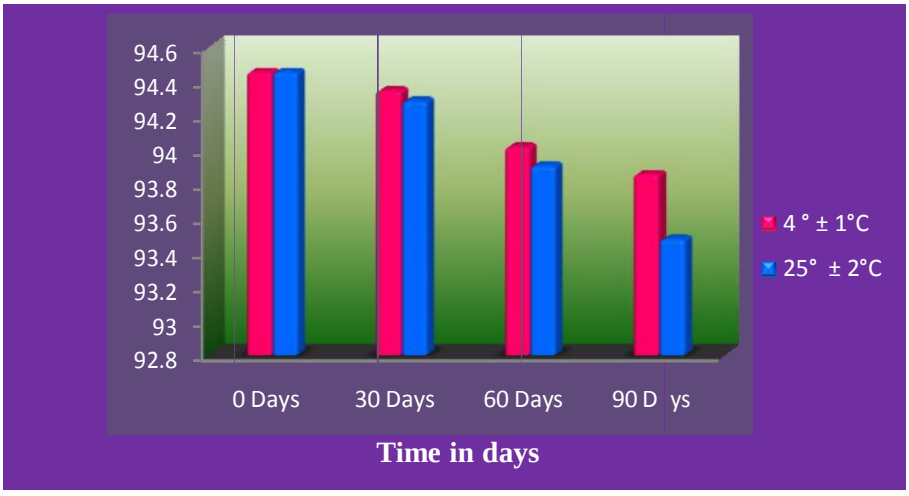


Fig. 9.43: Stability study (*In-vitro* drug release) of lyophilized nanoparticle formulation F6

Table 9.26: In-vitro drug release after 90days stability study of formulation F6

S.No.	Time / Temperature	*Percentage of *in-vitro* drug release	
		4 ± 1^0C	$25^0 \pm 2^0C$ / 60 ± 5 % RH
1	0 Days	94.46±0.57	94.46±0.57
2	30 Days	94.35±0.28	94.29±0.24
3	60 Days	94.02±0.16	93.91±0.18
4	90 Days	93.86±0.16	93.48±0.24

* All the values expressed as mean ± mean S.D., n=3

The percentage in-vitro drug release studies after 90 days of the stability testing at different storage conditions are shown in Table 9.26 and Figure 9.43. By comparing this data with initial data it was observed that there is no change in % percentage drug release.

SUMMARY

AND

CONCLUTION

10.SUMMARY AND CONCLUSION

The present study was aimed to formulate pH sensitive nanoparticle by using polymers Eudragit®-EPO and surfactant Pluronic®-F 68 in order to treat the viral infections an attempt was made to study the effect of Eudragit®-EPO and Pluronic®-F 68 on characterization of nanoparticle.

Literature survey on nanotechnology proved that the polymer and surfactant selected for the present study have good nanoparticle forming property there are some reports with this new combination of polymer and surfactant used for research work.

Preformulation study was carried out for the drug identification. The identification of drug was determined by melting point, solubility, λ max, FTIR and loss on drying. The values of the study obeys that procured drug was confirmed as acyclovir.

The present studies are focused on application of 3^2 factorial design for the formation of nanoparticle with desired particle size. The effect ratio of polymer and stabilizer were found to influence the particle size, drug content and entrapment efficiency.

pH sensitive nanoparticle of acyclovir were prepared by nanoprecipitation technique. The prepared nanoparticles were characterized for particle size, drug content and entrapment efficiency. All the characterized parameters shows that formulation F6 nanoparticle with desired size, high drug content and high entrapment efficiency.

The drug and polymer compatibility study was carried out by FTIR and DSC analysis. The data's showed that there was no interaction between drug and polymer used in formulation F6.

From the SEM analysis study the formulation F6 shows that the particles are smooth and spherical in shape.

Formulation F6 shows the zeta potential value about 30 mV. It can be concluded that formulation F6 shown to be stable in suspension as the surface charge prevents aggregation of particle.

In-vitro drug release studies closely indicate that formulation F6 was found maximum amount of drug released among nine formulations at the end of 13 hours.

The value of the *in-vitro* drug release was fitted with the kinetic modeling for all formulation using PCP disso software. As per the kinetic drug release modify the formulation F6 are released the drug by following zero order drug release.

Form the stability data it can be concluded there was no significant change in drug content, drug entrapment efficiency and *in-vitro* drug release. Hence the formulation F6 is stable formulation.

The overall studies concluded that formulation F6 composed of drug, Eudragit®-EPO and pluronic®-F 68 in the ratio of 1:1.5:1 showed satisfactory and better release profile. Hence the formulation F6 considered as best formulation.

FUTURE PROSPECTS

11. FUTURE PROSPECTS

The present study focused on acyclovir loaded self assembled pH sensitive nanoparticle preparation using Eudragit®-EPO and surfactant Pluronic®-F 68 the nanoparticle prepared by nanoprecipitation method. The prepared nanoparticles were evaluated by *in-vitro* parameters. Along with *in-vitro* studies there is a need to perform *in-vivo* studies using animal model.

In future *in-vivo* studies will be conducted to set *in-vitro in-vivo* correlation which is necessary for the successful formulation development.

In future the long term stability studies are required to know the shelf life of the prepared nanoparticle.

The various formulations will be developed with acyclovir using other polymer and stabilizer in future.

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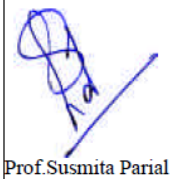

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